# PERSISTENCE OF LATE SV40 GENOME TRANSCRIPTION AFTER INHIBITION OF DNA REPLICATION BY CYTOSINE ARABINOSIDE

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## 1. Introduction

In SV40-infected African green monkey kidney (AGMK) cells the transcription of the early viral genes has been shown to be independent from the genome replication, whereas the transcription of the late viral genes is prevented by the presence of metabolic inhibitors of the DNA synthesis such as cytosine arabinoside (araC) [1,2]. This shows that the replication of the SV40 genome is a prerequisite for the initiation of the late viral genome transcription.

In the present paper the effect on the late SV40 genome transcription of araC added after the onset of the SV40 DNA replication was studied.

## 2. Materials and methods

### 2.1. *Materials*

The propagation of SV40 and of primary AGMK cells and the preparation of early and late SV40 specific RNA from infected cultures were performed as described elsewere [4].

### 2.2. *Hybridization techniques*

The hybridization with SV40 DNA according to the method of Gillespie and Spiegelmann [3] was performed as described elsewere [4]. Cellulose nitrate filters (23 mm, Sartorius, Göttingen) were used for competition hybridizations four pairs were used for each determination. SV40 DNA (form II) was immobilized on half of the filters, while the other half were blanks. Each of the pairs was incubated for 18 hr at 66°C in 1 ml of a solution containing 0.15 M NaCl, 15 mM sodium citrate, 0.1% sodium dodecyl sulfate, 1 mM EDTA, and unlabeled plus labeled RNA as indicated in the results. After the incubation the filters were washed, treated with RNase (20 $\mu g\ ml^{-1}$), and counted. The $^3H$-labeled and the unlabeled early lytical RNA were isolated from AGMK cells infected for 30 hr in the presence of 40 $\mu g\ ml^{-1}$ araC throughout the process so that genome replication did not occur.

## 3. Results

### 3.1. *Inhibition by araC of cellular and viral DNA replication in SV40 infected AGMK cells*

To be sure that in the transcription experiments the viral DNA replication was effectively suppressed, we first examined the inhibition, by araC, of [$^3$H]-thymidine incorporation into viral and host DNA. The rate of inhibition of the incorporation of [$^3$H]-thymidine into the total DNA (isolated according to [5]) was determined in SV40-infected and uninfected primary AGMK cells. We found that 40 $\mu g\ ml^{-1}$ araC suppresses the total DNA formation in infected cells by more than 98% (table 1). In addition, cell aliquots from similar experiments, layered on top of alkaline sucrose gradients, were centrifuged [6] and it was observed that the addition of araC also suppressed the incorporation of [$^3$H] thymidine into the viral DNA (fig. 1) (Some slowly sedimenting radioactive material to be independent of araC-treatment was found on top of all gradients). These data reveal that


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Table 1

| araC* ($\mu g \cdot ml^{-1}$) | SV40** | Specific radioactivity of Colter-DNA*** ($cpm \cdot \mu g^{-1}$) |
|---|---|---|
| – | – | $3.0 \times 10^3$ |
| 20 | – | $0.4 \times 10^3$ |
| – | + | $19 \times 10^3$ |
| 40 | + | $0.3 \times 10^3$ |

* Addition 48 hr p.i.
** 20 pfu per cell.
*** Labeling with 20 $\mu Ci \cdot ml^{-1}$ [$^3$H]thymidine from 48.5–49.5 hr p.i.

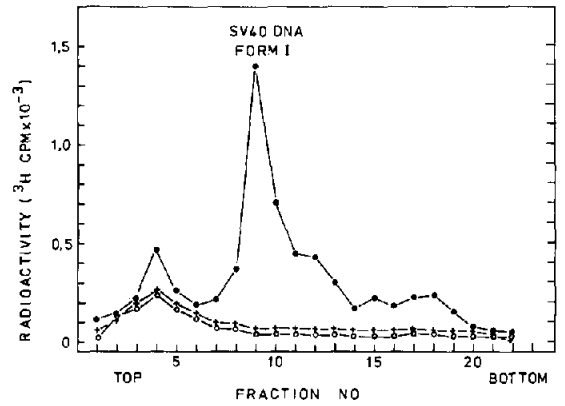


Fig. 1. [$^3$H]DNA profiles of SV40-infected, araC-treated AGMK cells. Petri dish (5 cm) monolayers of primary AGMK cells were infected with SV40 (10 pfu per cell), treated with araC (40 $\mu g \cdot ml^{-1}$) from 48 to 50 hr p.i., and labeled with [$^3$H]thymidine (10 $\mu Ci \cdot ml^{-1}$) from 49 to 50 hr p.i. – Then $1.9 \times 10^6$ cells were layered on top of an alkaline sucrose gradient [6] and spun for 5 hr at 4°C in a Spinco rotor SW 27 at 27 000 rpm. Fractions were collected from the tube bottom and counted in Insta Gel (Packard Instruments). •——• infected untreated cells. o——o Infected-araC treated cells. +——+ Uninfected-araC treated cells.

the determination of the late SV40 transcription reported below was carried out after host and viral DNA replication was effectively inhibited.

### 3.2. *Rate of incorporation of [$^3$H]uridine into SV40-specific RNA after interruption of DNA replication by araC*

The rate of [$^3$H]uridine incorporation into newly synthesized SV40-specific RNA was determined at three different time periods after the inhibition of the DNA replication with araC. The total RNA was isolated and 2 $\mu g$ of SV40 DNA hybridized with 1 $OD_{260}$ unit of RNA as described elsewere [4]. After adding araC, the rate of SV40 transcription was found to be unaffected for some hours, but then decreased gradually (fig. 2).

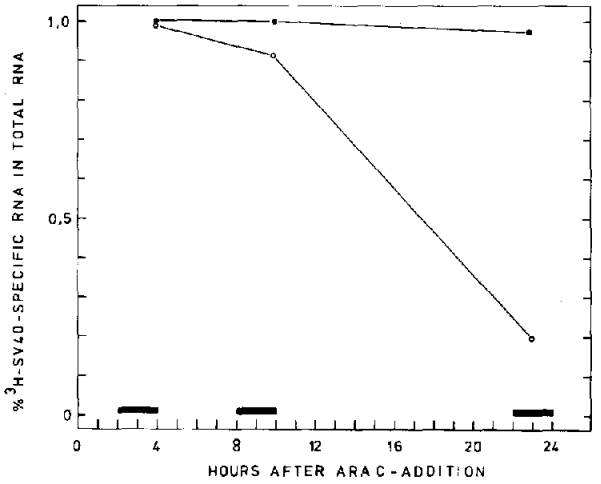


Fig. 2. Formation of SV40 specific RNA at different times after addition of araC. AGMK cells were infected with 10 pfu SV40 per cell. 48 hr later araC (40 $\mu g \cdot ml^{-1}$) was added or not added. At three different times, aliquots of the cultures were labeled with [$^3$H]uridine (50 $\mu Ci \cdot ml^{-1}$) for 2 hr. The SV40-specific RNA content of the cultures was determined by filter hybridization with SV40 DNA [3,4]. o——o AraC treated infected cells. •——• Untreated infected cells. ▬▬ [$^3$H]uridine labeling periods (2 hr).

### 3.3. *Persistence of late SV40 genome transcription after the inhibition of DNA replication*

Competition experiments were performed to determine the ratio of late SV40 RNA within the total $^3$H-labeled RNA pool. It was found that whether or not DNA replication had been inhibited, the hybridization of $^3$H-labeled SV40-specific RNA could be competed both by unlabeled late lytical SV40 RNA (90–95% competition) and by unlabeled early SV40-specific RNA (40% competition). Control experiments showed that the hybridization of labeled early SV40 RNA was competed to a great extent (95%) by unlabeled early SV40 RNA.

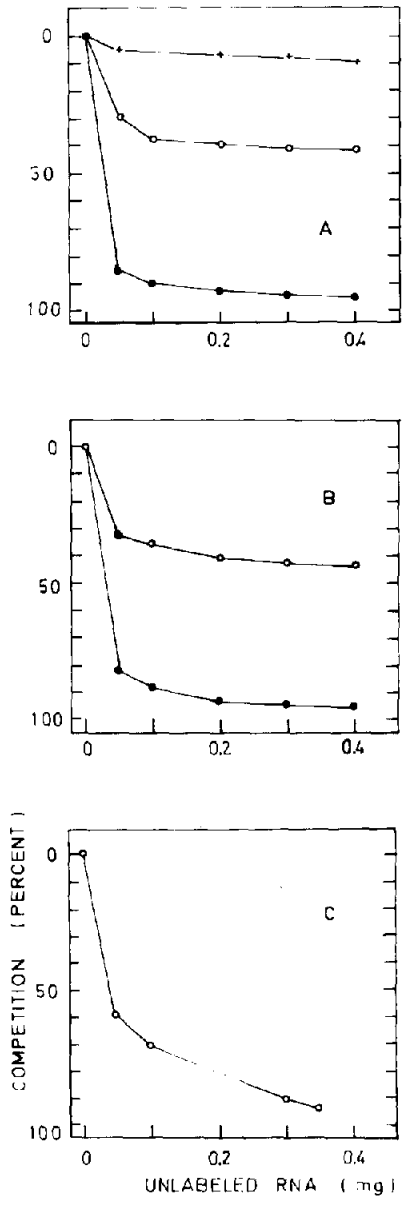


Fig. 3. Detection of late $^3$H-labeled SV40 specific RNA by competition hybridization: $^3$H-RNA for hybridization: 4 μg (A and B) and 60 μg (C). 0% competition corresponds to 910 cpm (A), 630 cpm (B), and 830 cpm (C). A: $^3$H-late SV-40 RNA vs. unlabeled early and late SV40 RNA, and RNA from uninfected control cells. B: $^3$H-SV40 RNA, labeled from 2 to 4 hr after araC addition vs. early and late unlabeled SV40 RNA. C: (Control) $^3$H-early SV40 RNA vs. unlabeled early SV40 RNA. Competing RNAs: +——+ Unlabeled RNA from uninfected cells. o——o Unlabeled early SV40 RNA. •——• Unlabeled late SV40 RNA.

## 4. Discussion

The findings indicate that an interruption of the SV40 DNA replication by the metabolic inhibitor araC does not impair either the viral transcription rate or the transcription of the late virus specific sequences if this interruption occurs after DNA replication has begun.

The slow decrease of the transcription rate which occurs several hours after the addition of araC could be explained assuming a decrease of the template concentration caused by the continuing encapsidation of the viral DNA. This explanation is based on the speculation [7] that the late genes are transcribed either from newly synthesized free DNA molecules by a modification of host cell RNA polymerase II or from DNA molecules which contain a promotor.

A similar independence from a current DNA replication of the continous late genome transcription is also known in the case of adenovirus [8] and of the SV40 mutant ts 30 when cells infected at the permissive temperature are shifted to the nonpermissive temperature [9].

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