Proc. Nat. Acad. Sci. USA
Vol. 70, No. 6, pp. 1654–1658, June 1973

"Early" Virus-Specific RNA May Contain Information Necessary for Chromosome Replication and Mitosis Induced by Simian Virus 40

(early SV40-specific RNA/tumor antigen/mitogen)

EVELYNE MAY, PIERRE MAY, and ROGER WEIL*

Institut de Recherches Scientifiques sur le Cancer, 94-Villejuif, France; and *Department of Molecular Biology, University of Geneva, 1211 Geneva, Switzerland

Communicated by V. Prelog, March 15, 1973

ABSTRACT **Simian Virus 40 (SV40) induces in "contact-inhibited" tissue culture cells of mouse kidney an abortive infection that leads to the appearance of intranuclear SV40-specific tumor (T-) antigen, followed by replication of the mouse-cell chromatin and mitosis, while no viral progeny DNA or capsid protein is produced. Synthesis of "early" SV40-specific RNA ("19S RNA") begins a few hours before the appearance of T-antigen and appears to be switched off after the onset of chromatin replication. As the most simple working hypothesis that can account for the experimental results available, we assume that early SV40 RNA contains information necessary for production of T-antigen and that this antigen (or an unknown early virus-specific function that would simply parallel the appearance of T-antigen) activates or de-inhibits a cellular regulatory element that governs chromosome replication and mitosis. The experimental results agree with the idea that SV40 acts primarily as a mitogen.**

Several lines of experimental evidence suggest that Simian Virus 40 (SV40) and polyoma virus act primarily as mitogens (i.e., inducers of chromosome replication and mitosis) and that their mitogenic action may be mediated by an activator, specified by an "early" viral gene(s) (1–3).

SV40 induces in "contact-inhibited" tissue culture cells of mouse kidney (MK) an abortive infection (4, 5) that leads to the appearance of intranuclear tumor (T-) antigen, followed by replication of the mouse-cell chromatin and mitosis (2, 3) while no detectable amounts of viral progeny DNA or capsid protein are produced (2, 5). The mouse kidney–SV40 system is therefore particularly useful study to the early functions of SV40.

In this paper we present some results from a long-term study on the mode of synthesis of "early" SV40 RNA and on its molecular and biological properties.

MATERIALS AND METHODS

SV40 used in these studies was grown and assayed for plaque-forming titer on CV-1 (monkey kidney) cell cultures (6). Viral lysates were obtained by infection of CV-1 cultures with very dilute viral preparations (0.01–0.1 PFU per cell).

Primary MK cell cultures (7), prepared from 10-day-old CR-1 mice, were grown in large plastic petri dishes (100-mm diameter), unless indicated otherwise, and were used for infection *3 days* after they had reached confluence. Under the conditions used (2, 3), confluent cultures contained about *10^5 cells per cm^2*. The cultures were infected with 0.4 ml of crude SV40 viral lysate or dilutions thereof. In all experiments the virus was adsorbed to the cells at 37° for 1 hr in a CO_2 incubator. The beginning of the adsorption period is considered as *time zero of the infection*. After adsorption, the cultures were washed once and then covered with *serum-free* reinforced Eagle's medium warmed to either 37° or 27° (2). In all experiments, control cultures were mock-infected and then treated in the same way as SV40-infected cultures. For all experimental points reported, at least two or three SV40-infected or mock-infected cultures were used. In experiments where DNA synthesis was to be inhibited with FdU (5-fluorodeoxyuridine, Hoffman-La Roche; 15 μg/ml) or ara-C (cytosine arabinoside, Sigma; 20 μg/ml), the inhibitor was present in the medium used to cover the cultures after the adsorption period. In MK cultures infected under standard conditions with viral lysates containing 1 to 2 × 10^8 PFU/ml, FdU decreased the incorporation of [*5*-^{3}H]-deoxycytidine (CEA-France; 16 Ci/mmol, 5 μCi/ml) by 85% while ara-C inhibited the incorporation of [^{3}H]dT ([*5*-^{3}H]methyl-thymidine; CEA-France; 12–20 Ci/mmol, 5 μCi/ml) by 95%, as determined by 1-hr pulses at various times between 6 and 33 hr after infection.

Autoradiography for determination of the number of DNA-synthesizing cells and immunofluorescence tests to detect intranuclear SV40-specific T-antigen were performed as described (2).

To obtain RNA labeled with [^{3}H]U ([*5*-^{3}H]uridine, CEA-France; 12–20 Ci/mmol) we added to SV40-infected and mock-infected MK cultures, at the times and for the periods indicated in *Results*, 2 ml per culture of warmed (37° or 27°) medium containing 400 μCi [^{3}H]U. If the infection took place in the presence of FdU or ara-C, the inhibitor was also present during the pulses with [^{3}H]U. Total (i.e., cellular and viral) RNA was extracted at the end of the [^{3}H]U pulses with hot phenol (8, 9).

SV40 to be used for RNA·DNA hybridization studies was purified in KBr gradients (10). SV40 viral DNA in the form of double-stranded cyclic coils (11) was obtained by sedimenting the purified viral preparations through alkaline CsCl solutions (pH 12.5) under the conditions used to prepare "53S" polyoma DNA (12). The double-stranded cyclic coils were converted into single strands by boiling for 30 min in 15 mM NaCl–1.5 mM Na citrate (pH 7.4), followed by quenching in ice water. The now fully denatured, single-stranded SV40 DNA was diluted in 0.9 M NaCl–0.09 M Na citrate and fixed by filtration to membrane filters (25-mm diameter; Schleicher and Schuell, BA 85). Filters, 3.5-mm diameter, were cut from the dried and baked (80°, 2 hr) membrane filters. Blank filters containing no DNA were treated and cut in the same way. RNA·DNA hybridization experiments

Abbreviations: SV40, Simian Virus 40; T-antigen, tumor antigen; PFU, plaque-forming units; MK, mouse kidney.

were generally performed either in urea (13) or in formamide (ref. 14 and B. Cavalieri, unpublished). The RNA preparations to be assayed for radioactive, hybridizable RNA (per assay, 90–120 μg of RNA containing 0.7 to 1.1 $\times 10^7$ cpm) were precipitated with two volumes of ethanol. The pellets were suspended in 100 μl of hybridization solution [7 M urea (Mann)–0.6 M NaCl–0.06 M Na citrate–0.1% sodium dodecyl sulfate (Sigma) pH 7.7, or 50% (v/v) formamide (Merck, chromatography grade)–0.3 M NaCl–0.03 M Na citrate–0.1% sodium dodecyl sulfate–0.01 M TES (TES = *N*-tris(hydroxymethyl) methyl-2-aminoethane sulfonic acid; Calbio, A Grade) pH 7.4] and were then transferred to 300-μl capacity capped polyethylene tubes (Beckman, Ref. 10123). To each tube two filters, each containing 0.2 μg of SV40 DNA and two blank filters, were added.

Hybridization in urea was performed at 41° for 5 days (first cycle). The four filters were then removed and replaced with two fresh DNA-containing filters and two fresh blank filters, which were incubated in the same hybridization mixture at 41° for 5 days (second cycle). *Hybridization in formamide* (one cycle only) was done at 37° for 70 hr. Under the conditions used, little if any viral DNA detached from the filters during the hybridization (unpublished). In some comparative experiments hybridization was performed in 0.6 M NaCl–0.06 M Na citrate at 65° (9). After hybridization the filters were washed once with 0.3 M NaCl–0.03 M Na citrate and then incubated at room temperature for 1 hr in 0.3 M NaCl–0.03 M Na citrate containing 20 μg/ml of pancreatic ribonuclease (Choay; incubated at 80° for 15 min). Then they were washed 6 times with 0.3 M NaCl–0.03 M Na citrate. The washed filters were dried. Scintillation fluid was added and the radioactivity was determined. Counting efficiency for tritium was 56.5%. The counting background, corresponding to 15 cpm, was subtracted from all results reported in this paper.

To obtain *"early"* SV40 RNA (Fig.5), we infected MK cultures with SV40 (6 $\times 10^8$ PFU/ml) at 37°, labeled with [^{3}H]U (500 μCi/ml; 20 Ci/mmol) from 10–16 hr after infection and then extracted with hot phenol; specific radioactivity of the MK RNA was 3.2 $\times 10^5$ cpm/μg. To obtain *"late"* SV40 RNA (Fig. 5), we infected CV-1 cultures with SV40 (6 $\times 10^8$ PFU/ml), labeled with [^{14}C]U (20 μCi/ml; 325 mCi/mmol) from 40–46 hr after infection and then extracted with hot phenol. Specific radioactivity of the CV-1 RNA was 3.2 $\times 10^4$ cpm/μg. For sedimentation, 115 μg of MK [^{3}H]RNA and 50 μg of CV-1 [^{14}C]RNA were mixed in 100 μl of buffer (10 mM triethanolamine, pH 7.4–50 mM NaCl–1 mM EDTA) and then layered on top of a (4 ml) preformed, linear (15–30%, w/v) sucrose density gradient, prepared with the same buffer. The sample was sedimented in a Spinco SW-56 rotor at 54000 rpm for 165 min at 20°. Fractions of four drops each were collected from the bottom of the tube. From each fraction, aliquots of 5 μl were removed to determine radioactivity of total RNA. The remainder of each fraction was adjusted to 50% (v/v) formamide–0.1% sodium dodecyl sulfate–0.3 M NaCl–0.03 M Na citrate–0.01 M TES and then hybridized with SV40 DNA.

RESULTS

SV40-infected and mock-infected MK cultures were labeled at 37° or 27° with [^{3}H]U at different times between 1 and 75 hr after infection and for periods varying between 0.5 and 7 hr. RNA was extracted with hot phenol and then assayed for radioactive, hybridizable RNA by hybridization in urea, in formamide, or in 0.6 M NaCl–0.06 M Na citrate at 65°, with SV40 DNA fixed on membrane filters and present in large excess (15). We define as radioactive, hybridizable RNA the difference [ribonuclease-resistant radioactivity (cpm) on 4 (or 2) DNA-containing filters minus ribonuclease-resistant radioactivity (cpm) on 4 (or 2) blank filters] obtained from 2 (or 1) cycles of hybridization either in urea or in NaCl–Na citrate at 65° (or in formamide). This difference, expressed as the percentage of total, acid-precipitable radioactivity present in the hybridization mixture is defined as the (relative) amount of radioactive, hybridizable RNA.

RNA from mock-infected MK cultures "hybridized" in urea or in NaCl–Na citrate at 65° with SV40 DNA to an extent of about 0.001% (50–150 cpm/10^7 cpm present in the hybridization mixture). Since a comparable "hybridization" was observed with highly purified polyoma viral DNA ("53S DNA") and also with DNA from bacteriophage SP50, we regard it as the nonspecific background of the hybridization method. Hybridization (2 cycles) in urea or in NaCl–Na citrate at 65° resulted in nearly exhaustive removal of the radioactive, hybridizable virus-specific RNA from the RNA extracts (15, and unpublished observations). While hybridization in formamide gave a very low nonspecific background (0.0001%, corresponding to 10–15 cpm/10^7 cpm), no SV40 RNA could be recovered during a second cycle of hybridization, and the relative amounts of radioactive, hybridizable SV40 RNA were always 25–50% lower than those determined by hybridization in urea or in NaCl–Na citrate at 65°.

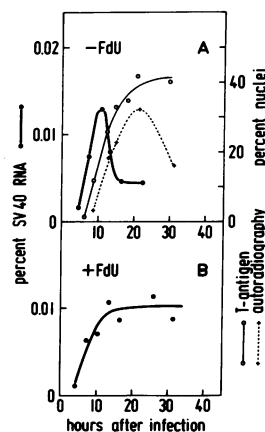

FIG. 1. (*A*) The temporal relation between synthesis of early SV40 RNA, T-antigen, and SV40-induced replication of cellular DNA during abortive infection at 37°. The values corresponding to SV40 RNA are the midpoints of 3-hr pulses with [^{3}H]U. The specific radioactivity of the phenol-extracted RNA varied between 6.0 and 9.0 $\times 10^4$ cpm/μg. The experimental points from mock-infected controls (0.0001%) are omitted. The values corresponding to autoradiography and T-antigen indicate the start of the 1-hr pulses with [^{3}H]dT and the time of fixation, respectively. In mock-infected cultures, the relative number of DNA-synthesizing cells varied between 3 and 4% (not shown). (*B*) Synthesis of early SV40 RNA under conditions where SV40-induced replication of cellular DNA is inhibited with FdU. The cultures were infected with SV40 and labeled with [^{3}H]U under the same conditions as in *A*. However, FdU was present in the medium from 1 hr after infection until the phenol extraction. The specific radioactivity of the phenol-extracted RNA varied between 6.6 and 8.5 $\times 10^4$ cpm/μg.

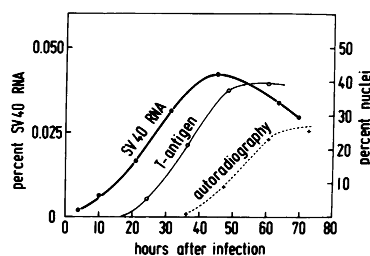

FIG. 2. Temporal relation between synthesis of early SV40 RNA, T-antigen, and SV40-induced replication of cellular DNA during the abortive infection at 27°. The values corresponding to SV40 RNA are the midpoints of 6-hr pulses with [^{3}H]U. The experimental points from mock-infected cultures (0.001% or less) are omitted. The specific radioactivity of the phenol-extracted RNA varied between 7.0 and 8.8 × 10^4 cpm/μg. Autoradiography and T-antigen as in Fig. 1A. In mock-infected cultures, the relative number of DNA-synthesizing cells varied between 1 and 2% (not shown).

Operationally, we defined the radioactive, hybridizable SV40 RNA produced (i.e., synthesized and processed) during the abortive infection as *"early" SV40 RNA*.

Temporal relation between early SV40 RNA and the subsequent events of abortive infection

Under Standard Conditions of Infection at 37°. MK cultures were infected at 37° with an SV40 viral lysate containing 2 × 10^8 PFU/ml. At the times indicated in Fig. 1A, aliquots of infected and mock-infected cultures were labeled for 3 hr with [^{3}H]U; RNA was extracted with hot phenol and then hybridized in formamide with SV40 DNA. The results in Fig. 1A are representative of six other, independent experiments performed under similar conditions.

Small amounts of radioactive, hybridizable SV40 RNA (0.0016%) could already be detected in the RNA extracted from cultures labeled between 3 and 6 hr after infection. Thereafter the amounts rapidly increased, reached a peak around 9–12 hr (0.0130%), and then rather abruptly decreased to a low plateau (0.0044%) which was maintained during the period of observation, i.e., at least until 30 hr after infection. Hybridization in urea gave analogous results.

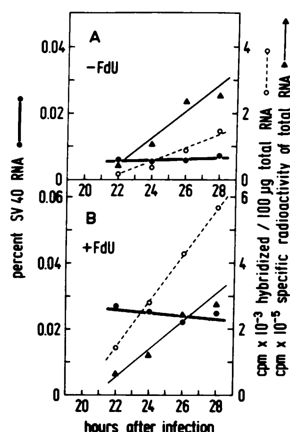

FIG. 3. Evidence suggesting that early SV40 RNA accumulates during the pulses with [^{3}H]U. [^{3}H]U was added 21 hr after infection. (A) Infection in normal medium. (B) Infection in medium containing FdU, added 1 hr after infection and present until phenol extraction.

On parallel cultures we studied the appearance of SV40-specific T-antigen, detectable by the immunofluorescence reaction, and, by the use of autoradiography (1-hr pulses with 5 μCi/ml of [^{3}H]dT) (2) the time course of SV40-induced cellular (chromosomal) DNA synthesis. In all experiments the increase of SV40 RNA preceded by 2–3 hr the appearance of T-antigen, while its subsequent decrease coincided in time with the asynchronous onset of SV40-induced cellular DNA synthesis (Fig. 1A).

During Infection at 27°. The abortive infection with SV40 proceeds at 27° similarly as it does at 37°. However, due to a cold-sensitive metabolic process ("psychrosensitive event") that is intercalated between the appearance of T-antigen and the onset of SV40-induced chromosome replication the time course is markedly slower at 27° (2, 3).

MK cultures were infected at 27° with an SV40 viral lysate containing 1.5 × 10^8 PFU/ml (Fig. 2). At the times indicated in the figure, aliquots of infected and mock-infected MK cultures were labeled with [^{3}H]U for 6-hr periods; RNA was then extracted with hot phenol and hybridized in urea with SV40 DNA.

Small amounts of SV40 RNA, corresponding to 0.0025%, could already be detected in RNA labeled from 1–7 hr after infection. Thereafter the amounts increased, reached a maximum of 0.040% around 40 hr after infection, and then slowly decreased. The increase in SV40 RNA preceded by 10 hr or more the appearance of T-antigen, while the subsequent decrease of SV40 RNA coincided in time with the rather slow, asynchronous onset of SV40-induced cellular DNA synthesis.

Decrease in early SV40 RNA may be linked to SV40-induced cellular DNA synthesis

In eight independent experiments we studied the time course of the synthesis of SV40 RNA under conditions where SV40-induced cellular DNA synthesis was inhibited with FdU (15 μg/ml) or ara-C (20 μg/ml), added 1 hr after infection. With both inhibitors, essentially the same results were obtained. Confirming earlier reports (16), we found that the time course of the appearance of T-antigen is indistinguishable in cultures infected in the presence or the absence of the inhibitors. Fig. 1B shows that in the presence of FdU the initial increase in SV40 RNA takes place just as it does in parallel cultures infected without the inhibitor (Fig. 1A),

TABLE 1. *Decrease in amounts of early SV40 RNA after the (synchronized) onset of SV40-induced cellular DNA synthesis (37°)*

FdU present (hr after infection)	dT present (hr after infection)	3-hr pulse with [^{3}H]U[a] (hr after infection)	Relative amounts of radioactive, hybridizable early SV40 RNA (%)[b,c] Exp. 1	Exp. 2
None	None	24–27	0.005	0.007
1–21	None	18–21	0.026	0.017
1–27	None	24–27	0.022[d]	0.024[f]
1–21	21–27	24–27	0.009[e]	0.008[g]

[a] 400 μCi/2 ml. [b] Hybridization in urea. [c] Mock-infected controls (±FdU) 0.001% or less. [d–g] Specific radioactivity (cpm/μg) of MK RNA (d = 6.0 × 10^4; e = 6.4 × 10^4; f = 1.2 × 10^5; g = 1.2 × 10^5.

but that thereafter the amounts of SV40 RNA remain high. It should be pointed out that synthesis and processing of *cellular RNA* are closely similar in cultures infected with SV40 in the presence or the absence of FdU or ara-C (manuscript in preparation).

In two other, independent experiments we studied the synthesis of SV40 RNA before and after release of the inhibition imposed by FdU (Table 1). MK cultures were infected at 37° with SV40 viral lysates containing 2×10^8 PFU/ml. The infection proceeded in the presence of FdU until the amounts of SV40 RNA had reached the high plateau. At 21 hr after infection, the FdU-containing medium was replaced with fresh medium containing 5 μg/ml of dT, a procedure that leads to the immediate and specific reversal of the inhibition imposed by FdU on DNA synthesis (17). The results in Table 1 show that by 6 hr after the (synchronized) onset of SV40-induced cellular DNA synthesis, the SV4O RNA had decreased to the small amount observed in parallel cultures infected for the same length of time in the absence of FdU. The results also show that the incorporation of [^{3}H]U into RNA remained virtually unchanged before and after addition of dT.

"Turnover" of early SV40 RNA

To determine whether the rapid decrease in the amounts of SV40 RNA observed after the onset of SV40-induced cellular DNA synthesis was due to an increased catabolism or a decreased rate of synthesis of early SV40 RNA, we performed the following experiments.

"Cumulative" Labeling with [^{3}H]U. The results shown in Fig. 3 are representative of two other, independent experiments. MK cultures were infected at 37° with an SV40 viral lysate containing 2×10^8 PFU/ml. One set of cultures was covered 1 hr after infection with normal medium and the other set with medium containing FdU. The infection then proceeded until the SV40 RNA had reached the low plateau in the cultures infected under standard conditions and the high plateau in those infected in the presence of FdU. At 21 hr after infection, fresh medium containing [^{3}H]U ($\pm$FdU) was added to all cultures. From both sets of cultures aliquots were extracted with hot phenol 1, 3, 5, and 7 hr later. We then determined for each RNA preparation specific radioactivity (cpm/μg RNA) and total amounts of radioactive, hybridizable SV40 RNA (cpm/100 μg RNA). The results show that both specific radioactivity of total RNA and the amounts of radioactive, hybridizable SV40 RNA increased linearly with the length of the [^{3}H]U pulse and that the relative amounts of SV40 RNA thus remained fairly constant.

"Pulse-chase" Experiments with [^{3}H]U. In three independent experiments, SV40-infected (1 to 2×10^8 PFU/ml) MK cultures were labeled for 1 hr with 500 μCi/ml of [^{3}H]U at different times during the ascending part, the peak, and the descending part of the curve corresponding to SV40 RNA, shown in Fig. 1*A*. From one set of cultures RNA was extracted with hot phenol immediately after the 1-hr pulse. The remainder of the labeled cultures was washed with and then incubated in nonradioactive medium (containing 10 μg/ml of uridine) for 2, 4, or 6 hr before phenol extraction ("chase"). The results show that under the conditions used and at the times tested (8–22 hr after infection), newly synthesized early SV40 RNA turned over rather slowly and at a similar rate with an estimated half-life of more than 2.5 hr (manuscript in preparation).

These observations suggest (*a*) that the turnover of newly synthesized early SV40 RNA is slow throughout the abortive infection, (*b*) that radioactive, hybridizable early SV40 RNA accumulated during the pulses with [^{3}H]U and, (*c*) that, as judged by the methods used, the turnover of early SV40 RNA is indistinguishable in cultures infected in the absence or the presence of FdU (or ara-C). We are therefore led to the tentative conclusion that, in a given experiment, the relative amounts of radioactive, hybridizable early SV40 RNA reflect the rates at which the cultures produced early SV40 RNA during the pulses with [^{3}H]U and, furthermore, that the decrease in the amounts of radioactive, hybridizable early SV40 RNA soon after the onset of SV40-induced cellular DNA synthesis (Fig. 1 and 2; Table 1) is due to a decrease or, more likely, a switch-off of transcription.

A linear relation between early SV40 RNA, the number of T-antigen-positive nuclei, and the logarithm of the plaque-forming titer of the viral preparations used for infection

The results shown in Fig. 4 are representative of two other, similar experiments. Aliquots of 6 MK cultures were infected at 37° with an SV40 viral lysate containing 2×10^8 PFU/ml or with 4-, 10-, and 40-fold dilutions thereof. 1 hr after in-infection, all cultures were covered with medium containing FdU. From each dilution group, two cultures were labeled with [^{3}H]U from 5–10 hr (pulse 1) and two other cultures from 10–15 hr (pulse 2). From the two remaining cultures of each dilution group coverslips were fixed 15 hr after infection for determination of the relative number of T-antigen-positive nuclei. At the end of each labeling period (i.e., at 10 and 15 hr after infection), the cultures were extracted with hot phenol. The two RNA preparations from pulse 1 and 2 of each dilution group were pooled, assayed for specific radioactivity, and then hybridized in urea with SV40 DNA. T-antigen and SV40 RNA could only be detected if the viral preparations used for the infection contained at least 10^6 PFU/ml. Thereafter both the relative number of T-antigen-positive nuclei and the amounts of radioactive, hybridizable SV40 RNA (produced between 5 and 15 hr after infection) increased linearly with the logarithm of the plaque-forming titer. These results suggest that abortively infected MK cells (which subsequently produce T-antigen) synthesize early SV40 RNA at a similar rate.

Sedimentation pattern of radioactive, hybridizable early SV40 RNA

MK cultures were infected with SV40 viral lysates (6×10^8 PFU/ml) and then labeled with 500 μCi/ml of [^{3}H]U for 0.5, 1, or 6 hr at different times between 6 and 16 hr after infection. RNA was extracted with hot phenol at the end of the [^{3}H]U pulses and then sedimented through linear (15–30%, w/v) sucrose density gradients. Fractions were collected and hybridized with SV40 DNA. Hybridization in formamide or 0.6 M NaCl–0.06 M Na citrate at 65° gave closely similar results. In all RNA preparations tested, the bulk of the radioactive, hybridizable early SV40 RNA sedimented as a rather uniform band (Fig. 5) with a sedimentation coefficient of about 19 S, while a small fraction consisted of slower sedimenting (about 16S) RNA molecules. In RNA from cultures that had been labeled for 0.5 hr, small amounts

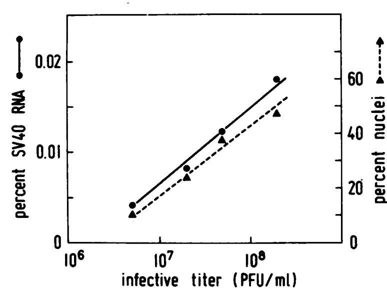

FIG. 4. Linear relation between early SV40 RNA, T-antigen, and the logarithm of the plaque-forming titer of the viral preparation used for the infection.

of SV40 RNA sedimented between 20 and 26 S. However, no evidence was obtained for the presence of virus-specific "giant" RNA molecules (9). Fig. 5 shows, furthermore, that the 19S early SV40 RNA overlaps with a species of SV40 RNA produced late during the lytic infection (18). It remains unknown whether 19S early SV40 RNA is synthesized as such or whether it is derived by very rapid cleavage and/or hydrolysis from larger precursor RNA molecules.

DISCUSSION

The results reported here suggest that the initial increase in the amount of radioactive, hybridizable early SV40 RNA, which precedes by a few hours the appearance of T-antigen (Figs. 1*A* and 2), is the expression of the asynchronous onset of the synthesis of early SV40 RNA and that the subsequent rapid decrease may reflect the switch-off of transcription, linked to SV40-induced replication of mouse cell chromatin.

The experimental results reveal a striking temporal and quantitative relation between early SV40 RNA ("19S RNA") and the subsequent events of the abortive infection. Though

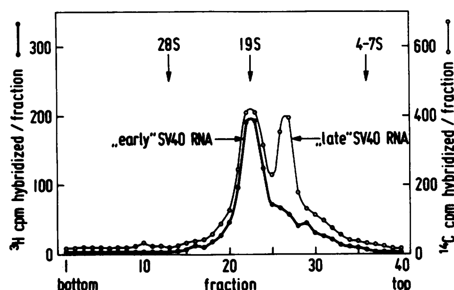

FIG. 5. Sedimentation pattern in a linear sucrose density gradient (15–30%, w/v) of a mixture of [^{3}H]U-labeled *early* SV40 RNA extracted from abortively infected mouse-kidney cell cultures and of [^{14}C]U-labeled *late* SV40 RNA extracted from lytically infected CV-1 (monkey kidney) cell cultures. MK cultures were labeled with [^{3}H]U from 10–16 hr and CV-1 cultures with [^{14}C]U from 40–46 hr after infection. The results shown in the figure are corrected for overlap of ^{14}C (23%) into the ^{3}H channel and for overlap of ^{3}H (0.1%) into the ^{14}C channel. The positions of cellular 28S ribosomal RNA and 4–7S RNA are indicated by *arrows*, while the position of the 18S ribosomal RNA is omitted. It should be pointed out that early and late SV40 RNA exhibit essentially the same pattern when sedimented separately (unpublished observations).

direct experimental evidence for a causative relationship is lacking, this possibility is indirectly supported by the observation that treatment of confluent MK cultures with mouse-specific interferon before (but not after) infection depresses by as much as 70% the synthesis of early SV40 RNA and results in a comparable reduction in the number of T-antigen-positive nuclei and in the number of cells participating in SV40-induced cellular DNA synthesis and mitosis (in preparation and refs. 15 and 19).

As the most simple working hypothesis that can account for the experimental results available, we assume that early SV40 RNA contains information necessary for production of SV40-specific T-antigen (see, however, *Discussion* in ref. 1) and that this antigen (or an as yet unknown early virus-specific function that would simply parallel the appearance of T-antigen) activates or de-inhibits a cellular regulatory element that governs chromosome replication and mitosis. The experimental results are thus in accordance with the idea that SV40 acts primarily as a mitogen.

We thank Dr. Klaus Scherrer for numerous useful discussions and for constructive criticism of this work. We thank Mrs Y. Coirault, S. Tlouzeau, and Miss J. Bordé for excellent technical assistance. These studies were supported by la Délégation Générale à la Recherche Scientifique et Technique, la Fondation pour la Recherche Médicale Francaise, la Ligue Nationale Francaise contre le Cancer, and by the Swiss National Foundation for Scientific Research (Grants 3.276.69 and 3.593.71).

1. Weil, R. & Kára, J. (1970) *Proc. Nat. Acad. Sci. USA* **67**, 1011–1017.
2. May, E., May, P. & Weil, R. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 1208–1211.
3. Weil, R., May, E., May, P. & Türler. H. (1973) in *Molecular Studies in Viral Neoplasia*, The University of Texas M. D. Anderson Hospital and Tumor Institute at Houston, 25th Annual Symposium on Fundamental Cancer Research (The Williams & Wilkins Co., Baltimore, Maryland), in press.
4. Black, P. H. & Rowe, W. P. (1963) *Proc. Soc. Exp. Biol. Med.* **114**, 721–727.
5. Kit, S., De Torres, R. A., Dubbs, D. R. & Salvi, M. L. (1967) *J. Virol.* **1**, 738–746.
6. Jensen, F. C., Girardi, A. J., Gilden, R. V. & Koprowski, H. (1964) *Proc. Nat. Acad. Sci. USA* **52**, 53–59.
7. Winocour, E. (1963) *Virology* **19**, 158–168.
8. Scherrer, K. (1969) in *Fundamental Techniques in Virology*, eds. Habel, K. & Salzman, N. P. (Academic Press, New York, London), pp. 413–432.
9. Acheson, N. H., Buetti, E., Scherrer, K. & Weil, R. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 2231–2235.
10. Swetly, P., Brodano, G. B., Knowles, B. & Koprowski, H. (1969) *J. Virol.* **4**, 348–355.
11. Weil, R. & Vinograd, J. (1963) *Proc. Nat. Acad.Sci. USA* **50**, 730–738.
12. Hudson, J. B. (1969) *Eur. J. Biochem.* **9**, 112–117.
13. Kourilsky, Ph., Manteuil, S., Zamansky, M. H. & Gros, F. (1970) *Biochem. Biophys. Res. Commun.* **41**, 1080–1087.
14. Krüger, R. & McCarthy, B. (1970) *Biochem. Biophys. Res. Commun.* **41**, 944–951.
15. May, E. (1972) Doctoral Thesis, University of Paris.
16. Rapp, F., Butel, J. S., Feldman, L. A., Kitahara, T. & Melnick, J. L. (1965) *J. Exp. Med.* **121**, 935–944.
17. Pétursson, G. & Weil, R. (1968) *Arch. Gesamte Virusforsch.* **24**, 1–29.
18. Weinberg, R. A., Warnaar, S. O. & Winocour, E. (1972) *J. Virol.* **10**, 193–201.
19. Gresser, I. (1972) *Advan. Cancer Res.* **16**, 97–139.