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Increased Rate of RNA Synthesis: Early Reaction of Primary Mouse Kidney Cells to Infection with Polyoma Virus or Simian Virus 40

ERICH PÖCKL AND ERHARD WINTERSBERGER*

Institute of Molecular Biology, University of Vienna, 1090 Vienna, Austria

The rate of transcription in isolated nuclei of primary mouse kidney cells increases by a factor of about 1.5 between 5 and 8 h after infection with polyoma virus or simian virus 40. This process requires intact virus and is inhibited by cycloheximide and actinomycin D. It appears to involve the activity of all three nuclear RNA polymerases, and evidence was obtained for an increase in the rate of synthesis of most, if not all, species of RNA that were already produced in resting cells before infection. The additional synthesis of a few new RNA species not made in uninfected cells, however, cannot be excluded. The increase in the rate of transcription seems to precede an increase in the rate of translation in infected cells. Our additional finding that large and small tumor antigens (T-antigens) are synthesized at the same time after infection suggests that these cellular reactions are early consequences of the action of one or both of these T-antigens in the infected cell. Experiments with simian virus 40 mutants provided strong evidence for an involvement of large T-antigen but not of small t-antigen. These studies furthermore indicate that the increase in the transcription rate is a prerequisite for the induction of DNA replication in simian virus 40-infected mouse kidney cells.

Primary cultures of kidney cells from 10-day-old mice are arrested in growth after reaching confluence. Infection of such cells with polyoma virus or simian virus 40 (SV40) leads to an induction of chromatin replication and mitosis, which, in the case of a lytic infection with polyoma virus, is accompanied by virus replication and followed by cell lysis (8, 30–32). Viral tumor antigen (T-antigen) can first be detected by immunofluorescence in a few cells about 12 h after infection and increases in amount up to 20 to 24 h, when nearly all cells appear T-antigen positive (33). DNA synthesis begins at about 15 h and reaches a maximum about 25 h after infection. At the same time there is a two- to threefold increase in the activity of cellular enzymes involved in various steps of DNA synthesis (9, 10, 14, 17, 37).

Although the detailed role of T-antigen in this cellular process is not known so far, several hypotheses are discussed. T-antigen could, for instance, be directly involved in the initiation of cellular DNA replication (3, 18–20), in analogy to the suggested role in viral DNA synthesis. Alternatively, it could function much earlier in the infection cycle by initiating cellular events which are later followed by the induction of the S-phase of the cell cycle (7, 15, 33, 36). Finally, it may well be that T-antigen is in fact involved in both types of cellular reactions.

To decide among the above possibilities it would be important to know which cellular reaction follows most closely the appearance of T-antigen in the infected cell. Early experiments by Weil and co-workers (25, 33) had already indicated an increase in the amount of cellular RNA shortly after the appearance of T-antigen (as measured by immunofluorescence). Although not dependent on ongoing DNA replication (the reaction did occur in the presence as well as in the absence of fluorodeoxyuridine), the time course of this increase closely resembled that of the induction of DNA replication (25). These results, therefore, do not clarify whether the increase in cellular RNA is a prerequisite for or a consequence of the initiation of DNA replication. Unfortunately, immunofluorescence is a rather insensitive method for the detection of T-antigen, and it is reasonable to assume that cellular reactions could be induced quite early after infection due to the activity of low quantities of T-antigen that are not yet demonstrable by immunofluorescence. Consequently we have become interested in events occurring in infected cells considerably earlier than the induction of DNA replication, and we report here that the capacity for cellular RNA synthesis increases in nuclei as soon as between 5 and 8 h after infection of primary mouse kidney cells with polyoma virus or SV40. We further-

more demonstrated, by immunoprecipitation and gel electrophoresis, that T-antigen is already synthesized at this early time of the infection cycle. Using mutants of SV40 we obtained evidence that large T- but not small t-antigen is required for the early increase in transcription rate, and that this increase is a prerequisite for the induction of DNA replication.

MATERIALS AND METHODS

Cell culture. Primary mouse kidney cell cultures were prepared from 10-day-old Swiss albino mice as described by Winocour (35) and grown in 85-mm plastic petri dishes with Dulbecco-modified Eagle medium containing 10% calf serum. One day after reaching confluence, cells were infected with either polyoma virus or SV40 at 5 to 50 PFU/cell. Mock-infected cells received medium in place of the virus suspension. After allowing 2 h at 37°C for virus adsorption, cells were covered with 10 ml of medium without serum. Wild-type polyoma virus (kindly obtained from R. Weil, Geneva) was multiplied and assayed at 37°C in primary mouse kidney cells. Wild-type SV40, the deletion mutants *dl*884 and *dl*890, the *tsA* mutant *tsA*209, and the double mutant *tsA*209-*dl*290 (kindly provided by R. Martin, Bethesda) were grown on the TC7 line, and plaque assays were carried out on the CV1 line of African green monkey cells. Except in the case of *tsA* mutants, which were grown and assayed at 32°C, virus growth and assays were performed at 37°C.

Isolation of nuclei and determination of transcription activity. Two plates were used for the isolation of nuclei at each time point (between 3 and 24 h after infection). Cells were trypsinized, collected, and washed in cold HEPES buffer (0.25 M sucrose, 25 mM HEPES [*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid], pH 8.0, 5 mM $CaCl_2$, 1 mM EDTA, and 30 mM 2-mercaptoethanol). Nuclei were prepared by the method of Bentz and Strominger (2), which involves gentle lysis of cells in isotonic buffers containing 0.25% Brij 58. These nuclei appear well preserved in phase-contrast microscopy and show high DNA- and RNA-synthesizing activity.

RNA synthesis was measured in 125-μl reaction mixtures containing 20 μl of nucleus suspension (2×10^5 to 4×10^5 nuclei per assay), 50 mM Tris-hydrochloride (pH 7.9), 1.6 mM $MnCl_2$, 2 mM $MgCl_2$, 60 mM $(NH_4)_2SO_4$, 0.5 mM dithioerythritol, 0.2 mM each of ATP, GTP, and CTP, 0.04 mM UTP, and 2 μCi of [^{3}H]UTP (specific activity 13 Ci/mmol). If assays were carried out in the presence of α-amanitin, the drug was added to a final concentration of 8 μg/ml. Reaction mixtures were incubated for 15 min at 37°C. RNA synthesis is linear within this period of time. RNA was then precipitated with 5% trichloroacetic acid (containing 1% sodium pyrophosphate) and collected on Whatman GF/C filters. These were washed with 5% trichloroacetic acid and methanol-ether (1:1) and then dried and counted in a liquid scintillation spectrometer.

In vivo labeling and analysis of RNA. RNA was labeled in mock- or polyoma virus-infected cells by adding [^{3}H]uridine (final activity, 10 μCi/ml of medium) or [^{14}C]uridine (final activity, 1 μCi/ml of medium). After a 90-min pulse, cells were washed twice with cold phosphate-buffered saline and removed from the plates by trypsinization. Total RNA was extracted with phenol-chloroform-isoamyl alcohol (12:12:1) at 65°C using a slight modification of the method of Scherrer (26). RNA was precipitated from the aqueous phase at −20°C with 67% ethanol–0.1 M NaCl and analyzed by electrophoresis in 0.5% agarose–2% polyacrylamide composite gels as described by Dingman and Peacock (6). After electrophoresis, gels were sliced, and the radioactivity in the slices was measured.

Extraction and analysis of RNA polymerases. Nuclei were prepared as described above using 24 plates of polyoma virus- or mock-infected mouse cells. RNA polymerases were extracted and chromatographed on columns (0.9 by 8.4 cm) of DEAE-Sephadex A 25 essentially as described (22). RNA polymerase activity was determined by the method outlined above using 25-μl portions of the eluate fractions. The salt concentration in various fractions was determined by conductivity measurement.

Labeling, extraction, and analysis of T-antigen. Mock-infected or SV40-infected mouse kidney cells were pulse-labeled for 2 h with [^{35}S]methionine (final radioactivity, 200 μCi/2 ml of methionine-free medium). After labeling, the cells were thoroughly washed with phosphate-buffered saline, and T-antigen was extracted, immunoprecipitated, and analyzed in polyacrylamide gels in the presence of sodium dodecyl sulfate by the method of Schwyzer (27).

DNA synthesis. DNA synthesis was measured by determining the amount of [^{3}H]thymidine incorporated into DNA during a 30-min pulse. [^{3}H]thymidine (1 μCi/ml of medium) was added to the cultures, which were then incubated at the temperature indicated in the various experiments. After 30 min, medium was removed, and the cells were washed twice with phosphate-buffered saline and lysed by addition of 1 ml of 0.1% sodium dodecyl sulfate in 0.01 M EDTA (pH 8) per petri dish (60-mm diameter). DNA was precipitated from the lysate with 10% trichloroacetic acid at 0°C, and the precipitate was collected on GF/C filters, which were then washed, dried, and counted.

RESULTS

The transcriptional activity of isolated nuclei increases early after infection. To avoid complications due to changes in the permeability of the cell membrane to uridine caused by virus infection, we have chosen to determine RNA synthesis rates in an in vitro assay system using isolated nuclei. The RNA synthesis capacity of isolated nuclei started to rise about 5 h after infection of primary mouse kidney cells with either polyoma virus or SV40 (Fig. 1). Rates of RNA synthesis reached a new level, about 1.5 times higher than that in mock-infected cells, around 8 h after infection and remained at this elevated level. Experiments using the inhibitor of RNA polymerase II, α-amanitin, indicated that the rate of both the synthesis of rRNA (due to RNA polymerase I) and that of mRNA (due to RNA polymerase II)

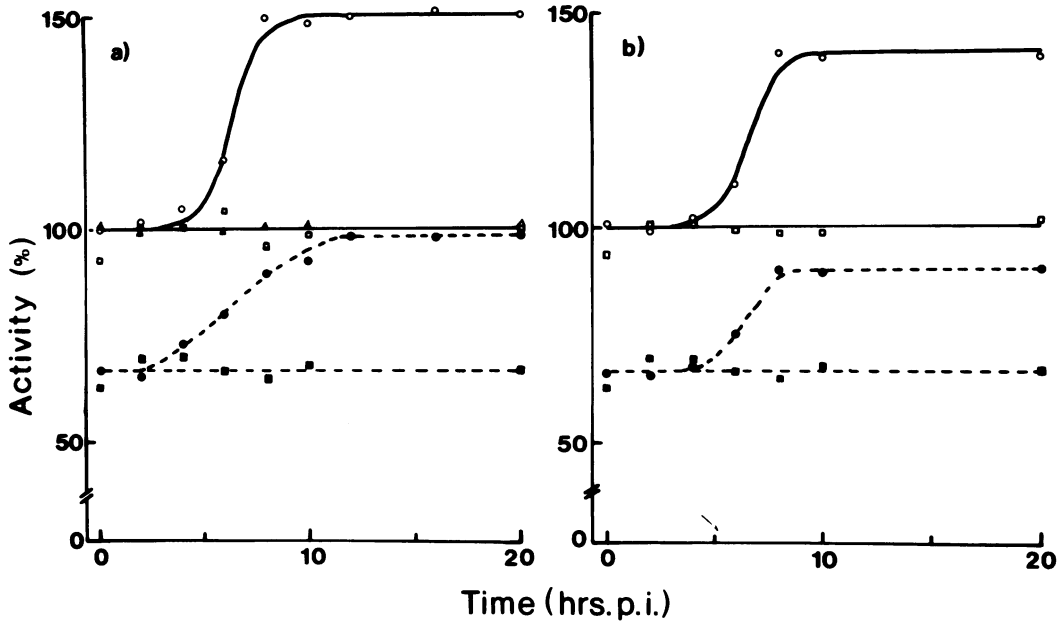

FIG. 1. *RNA synthesis in isolated nuclei from mouse kidney cells after infection with polyoma virus (a) or SV40 (b). Virus-infected cells (○); mock-infected cells (□); cells infected with UV-inactivated polyoma virus (△). Open symbols, Values obtained in the absence of α-amanitin; full symbols, those obtained in the presence of α-amanitin (8 μg/ml). 100% equals 3,860 cpm incorporated per 10^6 nuclei in (a) and 3,708 cpm incorporated per 10^6 nuclei in (b).*

increased to about the same extent. A rise in the activity of RNA polymerase III was not obvious from these experiments; however, other data to be described below are in accord with the assumption that the activity of all three nuclear RNA polymerases is in fact elevated in virus-infected cells. Infection with UV-inactivated polyoma virus did not result in any increase of the transcription rate in isolated nuclei (Fig. 1). This result indicates that a viral function is necessary for the observed increase in transcriptional activity. Support for this assumption comes from the observation that cycloheximide (20 μg/ml) or actinomycin D (4 μg/ml), added 2 h after virus infection, also caused the transcription rate to remain at the level of uninfected cells (Table 1). Both inhibitors blocked the expression of early viral functions (synthesis of T-antigen) in the infected cells.

All three nuclear RNA polymerases appear to be involved. Elevated activities of all three nuclear RNA polymerases could be caused, for instance, by modification of one of the subunits common to all three enzymes (see reference 4 for review). If this reaction involved a change in the charge of the enzymes, it might result in a recognizable shift in the elution profile of the enzymes from ion-exchange columns. Such a shift was observed by Mauck (20) in the case of RNA polymerase I of serum-stimulated 3T6 cells. We have tested this possibility by extracting RNA polymerases from infected and uninfected cells and chromatographing the enzyme preparations under identical conditions on columns of DEAE-Sephadex. By carefully monitoring the salt concentration in eluate fractions by conductivity measurement, we found that the peak fractions of enzymes from uninfected and infected cells appeared reproducibly at identical ionic strengths (Fig. 2). This was particularly evident for RNA polymerase I (fractions 19 to 24), whereas minor changes in the charge of a

TABLE 1. *Effect of cycloheximide and actinomycin D on the increase of the rate of RNA synthesis in polyoma virus-infected mouse kidney cells*[a]

Cells	Transcriptional activity (%)
Mock-infected cells	100
Polyoma virus-infected cells	
Without inhibitor	144
Plus cycloheximide	101
Plus actinomycin D	104

[a] Cycloheximide (20 μg/ml) or actinomycin D (4 μg/ml) was added to the cultures 2 h after infection. Nuclei were isolated 10 h after infection, and RNA synthesis rates were measured as described in the text; 100% equals 3,650 cpm incorporated per 10^6 nuclei.

fraction of RNA polymerase II (fractions 26 to 33) cannot be excluded because of the broadness of the peak of enzyme activity. The same holds for fractions containing RNA polymerase III activity (fractions 35 to 39).

Our assay system using intact nuclei most likely measures only rates of elongation of preinitiated RNA chains. We therefore assume that the increase in activity that we observe is due to an increase in elongation rates and not in more frequent initiation events. This possibility can be tested by measuring transcription rates in the presence of Sarkosyl or of high concentrations of salt (11, 13). These conditions release most or all of the constraints on the synthesizing activity of RNA polymerases on chromatin without removing the enzymes from the DNA. If infection with virus caused an increase in the number of RNA polymerase molecules bound to chromatin, this increase should persist if the activities are measured in the presence of high salt concentrations or of Sarkosyl. If, on the other hand, the increased transcription rate is due to faster elongation only, such an increase in the number of bound RNA polymerase molecules need not be demonstrable within the period of our assays. We examined this question and found that no increase in the amount of bound RNA polymerases could be observed (Table 2). On the contrary, the activity measured in the presence of Sarkosyl or of high salt seemed to remain unchanged in nuclei of mock- or virus-infected

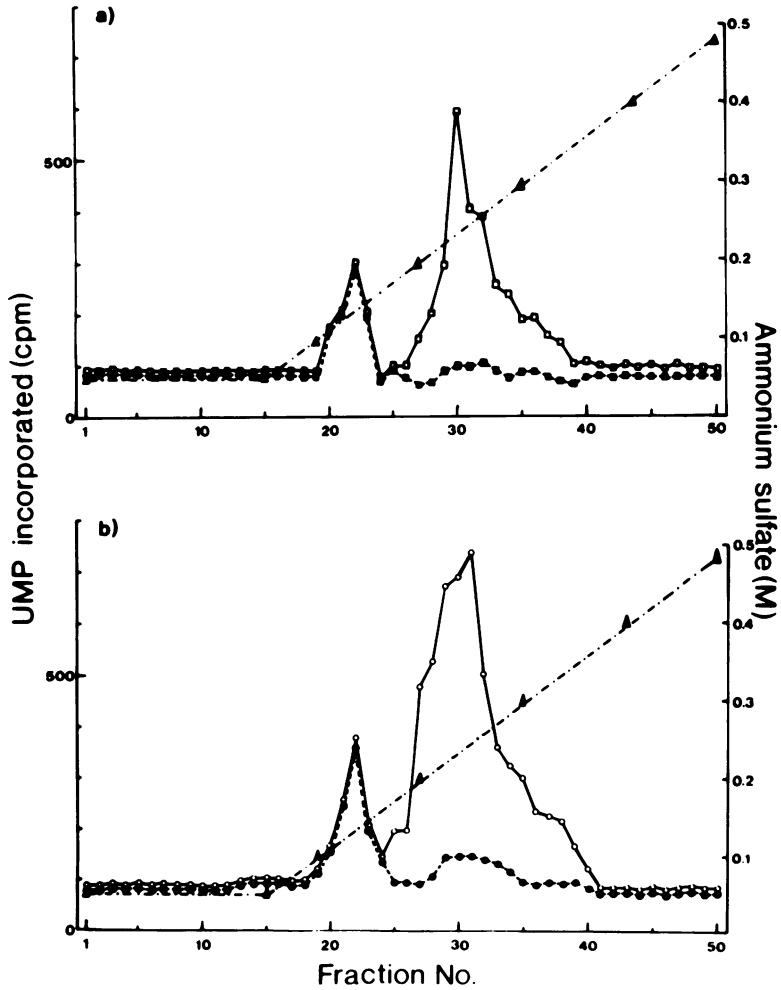

FIG. 2. *Chromatography of RNA polymerases on DEAE-Sephadex. (a) RNA polymerases extracted from nuclei of uninfected mouse cells (□, ■); (b) RNA polymerases extracted from nuclei of cells 8 h after infection with polyoma virus (○, ●). Open symbols, Activity in absence of α-amanitin; full symbols, activity in the presence of α-amanitin. (▲) Ammonium sulfate concentration.*

cells. This result is in accord with observations on growing and resting 3T3 cells (11) and indicates that it is not the number of bound enzyme molecules that is responsible for the elevated transcription capacity of nuclei isolated from infected cells. Initiation of RNA chain synthesis can be directly assayed by determining the level of incorporation of γ-^{32}P-labeled GTP at the 5′ position relative to the incorporation of [^{3}H]-UMP at internal positions of the RNA chains. We have performed such experiments and, in agreement with the Sarkosyl data mentioned above, have found that our transcription system is rather deficient in initiating RNA chains. On the average, only one in about 5,000 nucleotides is incorporated into the 5′ position, and this small amount does not change significantly if nuclei are isolated from infected cells.

Infection causes a general stimulation of total RNA synthesis. Our observation that all three nuclear RNA polymerases increase in activity in virus-infected cells indicates a rather general stimulation of total RNA synthesis and not the induction of only a few specific RNA species. This is supported by results of an experiment shown in Fig. 3. RNA synthesized in virus-infected cells was compared with that produced in uninfected controls. Polyoma virus-infected mouse cells were labeled for 1 h with [^{14}C]uridine (starting 7 h after infection), while mock-infected cells were at the same time labeled with [^{3}H]uridine. Cells from both cultures were then mixed, and the RNA was extracted and analyzed by gel electrophoresis. The ratio of ^{3}H to ^{14}C in slices did not deviate from the mean by more than 10%, indicating that infection primarily causes a more rapid synthesis of those RNA species that are also made in uninfected cells. From another identical experiment (not shown) in which RNA was analyzed under conditions including the complete 4-5S region in the gel, it is clear that these RNA species increase to the same extent as those of higher molecular weight.

Changes in the rate of translation in infected cells. We also determined rates of translation at different times after infection of primary mouse kidney cells with polyoma virus. By pulse-labeling cells with [^{3}H]leucine, we observed that the rate of protein synthesis started to increase somewhat later (around 7 h) after infection than that of RNA synthesis (Fig. 4). From this we tend to conclude that the stimulation of RNA synthesis precedes that of protein synthesis.

Is T-antigen responsible for the early increase in transcriptional rates? Since T-antigen can be demonstrated by immunofluorescence only considerably later in the infection cycle than the reaction described above, we used a different, more sensitive method to search for T-antigen in SV40-infected mouse cells at times when the first cellular reactions to the infection process are apparent. This method (27) involved labeling proteins with [^{35}S]methionine of high specific activity, followed by extraction, specific immunoprecipitation with hamster anti-T serum, and electrophoretic analysis of immunoprecipitates. The results of such an experiment are shown in Fig. 5. Large (90K) T-antigen could be clearly demonstrated in cells labeled between 6 and 8 h after infection. In fact, at 4 to 6 h after infection a protein band, identical with 90K T-antigen immunoprecipitated from lytically infected TC7 cells, but slightly different from a protein also present in mock-infected controls, could already be seen. The gels furthermore demonstrated that small (20K) t-antigen of SV40 (5, 23) appears to be synthesized in infected cells in parallel with large T-antigen. This raises the question of which one of the two T-antigens is responsible for the early increase in transcription rates.

Large T-antigen is required for the early increase in transcription rate of SV40-infected mouse kidney cells. We used two classes of SV40 mutants to decide which T-antigen is required for the early increase in transcription rate in infected mouse kidney cells. One class, exemplified by mutants *dl*884 and *dl*890, contains deletions between 0.535 and 0.600 units of the SV40 map (29). These mutants synthesize aberrant or no small t-polypeptides, but have normal large T-antigen (5, 29). The second class is mutants that are temperature sensitive in the *A* gene, which codes for large T-antigen (reviewed in 30, 31). These mutants do not support virus replication in the lytic system at the nonpermissive temperature due to a temperature-sensitive large T-antigen.

Table 3 summarizes the effect of an infection of primary mouse kidney cells with mutants *dl*884, *dl*890, or *tsA*209 on the rate of RNA

TABLE 2. *Effect of high salt or Sarkosyl on RNA polymerase activity in nuclei*[a]

Addition	Mock-infected cells		Polyoma virus-infected cells	
	− α-Amanitin	+ α-Amanitin	− α-Amanitin	+ α-Amanitin
No addition	4,351	2,583	6,962	4,079
Plus $(NH_4)_2SO_4$	10,145	2,348	11,346	3,264
Plus Sarkosyl	35,536	19,694	33,165	17,980

[a] Nuclei from mock-infected or polyoma virus-infected mouse kidney cells were incubated as described in the text except that, where indicated, $(NH_4)_2SO_4$ or Sarkosyl was added to a final concentration of 0.4 M or 0.1%, respectively. Numbers represent counts per minute of [^{3}H]UMP incorporated per 10^6 nuclei.

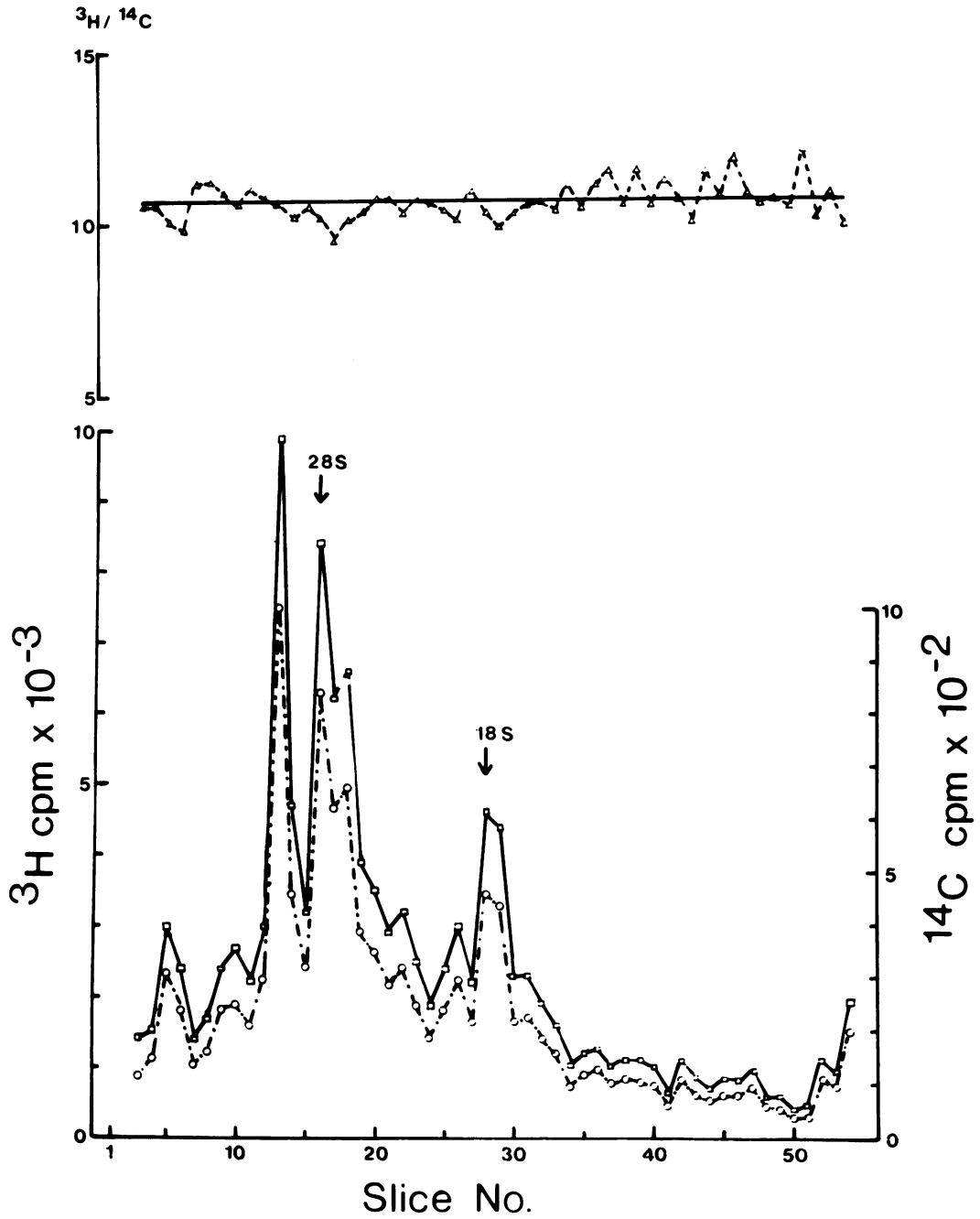

FIG. 3. *Analysis of RNA synthesized in mock-infected and polyoma virus-infected mouse kidney cells. Mock-infected cells (□) were labeled for 1 h with [^{3}H]uridine; virus-infected cells (○) were labeled with [^{14}C]uridine (7 h after infection). Cells from three plates each were then collected, mixed, and lysed in sodium dodecyl sulfate-containing buffer, and the RNA was extracted and analyzed by electrophoresis in an agarose-polyacrylamide gel.*

synthesis in isolated nuclei. Nuclei were prepared 10 h after infection at 37°C in case of the deletion mutants and either 24 h after infection at 32°C (the permissive temperature) or 10 h after infection at 39.5°C (the nonpermissive temperature) in the case of the *tsA* mutant. Mock-infected cells and cells infected with wild-type virus served as controls and were treated in the

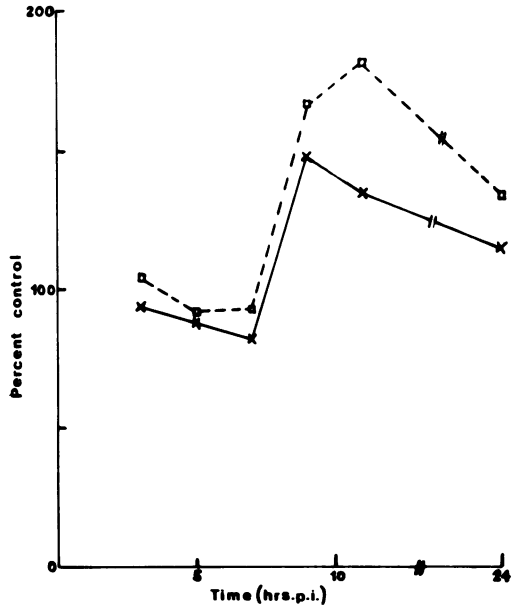

FIG. 4. *Change in the rate of protein synthesis in virus-infected mouse kidney cells. Primary mouse kidney cells (in 60-mm petri dishes) were infected with polyoma virus (×) or SV40 (□). Rates of protein synthesis were determined at various times after infection by adding [^{3}H]leucine (to a final radioactivity of 2 μCi/ml of medium) to the cultures. After a 30-min pulse, medium was removed, and the cells were washed extensively with cold phosphate-buffered saline. The cells were then lysed by addition of 1 ml of 20 mM EDTA (pH 7.5) containing 0.1% sodium dodecyl sulfate, and proteins were precipitated with 10% trichloroacetic acid. Precipitates were collected on membrane filters, washed, dried, and counted. Results are presented as percentage of translation rates in infected cells compared with those in mock-infected cultures used as controls.*

same manner as mutant-infected cells. The results of these experiments were quite clear: mutants *dl*884 and *dl*890 behaved like wild-type virus, whereas mutant *tsA*209 (the double mutant *tsA*209-*dl*290 gave the same result) led to an increase in transcription rate at 32°C but not at 39.5°C. This indicates that the early cellular reaction is a consequence of the activity of large SV40 T-antigen, whereas small t seems not to be involved.

We next asked the question whether this increase in transcription rate is a prerequisite for the induction of cellular DNA synthesis in SV40-infected primary mouse kidney cells. To answer this question, DNA synthesis was measured at various times after infection with mutants *dl*884 and *dl*890 (at 37°C) or *tsA*209 (at either 32°C or 39.5°C) (Fig. 6 and 7). In complete agreement with the results on the effect on transcription rate, it was found that deletion mutants induced DNA synthesis like wild-type SV40, whereas for the *tsA* mutant this was only true at the permissive, not at the nonpermissive temperature. These observations suggest a functional dependence of the initiation of DNA synthesis on a prior activation of transcription. Such an interpretation is supported by the experiment depicted in Fig. 8. Here cells were infected with the *tsA* mutant at 32°C, and after 24 h (when the early increase in transcription rate had taken place) they were shifted to 39.5°C. Under these

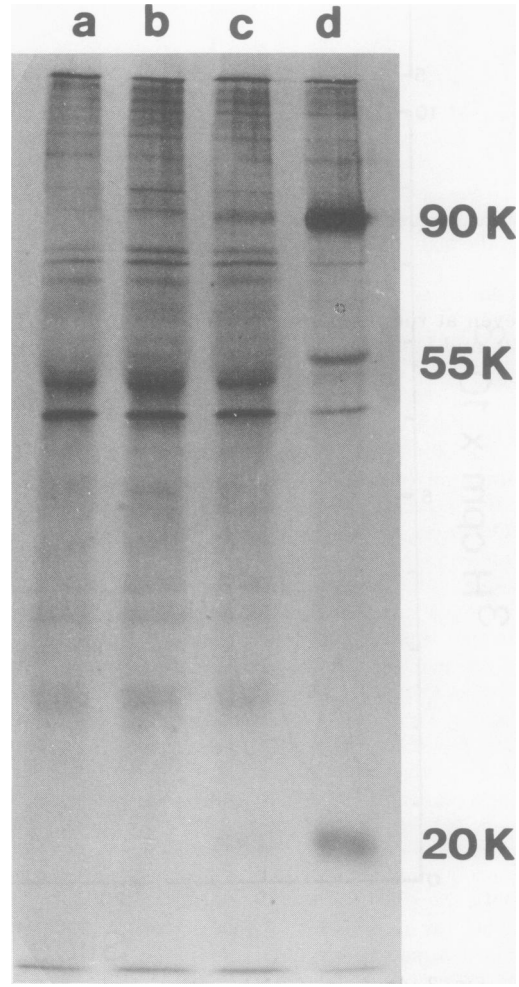

FIG. 5. *Characterization of [^{35}S]methionine-labeled proteins immunoprecipitated by antiserum against SV40 T-antigen. (a) Mock-infected cells; (b) SV40-infected cells labeled from 4 to 6 h after infection; (c) SV40-infected cells labeled from 6 to 8 h after infection; (d) T-antigen immunoprecipitated from TC7 cells lytically infected with SV40 and labeled between 25 and 30 h after infection.*

TABLE 3. *Induction of increased rate of RNA synthesis by infection of mouse kidney cells with SV40 mutants*[a]

Condition of infection	Temp of infection	Nuclei prepared at time (h p.i.):	[^{3}H]UMP incorporation (cpm/10^6 nuclei)	
			− α-Amanitin	+ α-Amanitin
Mock infection	37°C	10	4,704	3,850
Wild-type SV40	37°C	10	6,634	5,483
SV40 *dl*884	37°C	10	6,590	5,580
SV40 *dl*890	37°C	10	6,770	5,730
Mock infection	32°C	24	4,580	3,675
Wild-type SV40	32°C	24	6,535	5,403
SV40 *tsA*209	32°C	24	6,752	5,968
Mock infection	39.5°C	10	4,859	2,807
Wild-type SV40	39.5°C	10	7,273	4,280
SV40 *tsA*209	39.5°C	10	5,021	2,950
Mock infection	24 h at 32°C, then 4 h at 39.5°C		4,750	3,920
Wild-type SV40	24 h at 32°C, then 4 h at 39.5°C		6,745	5,750
SV40 *tsA*209	24 h at 32°C, then 4 h at 39.5°C		6,620	5,800

[a] Primary mouse kidney cells were infected by virus and then incubated at the temperature indicated. Nuclei were prepared at the indicated times postinfection (p.i.), and RNA synthesis was measured at 37°C as described in the text.

conditions the transcription rate remained high even at the nonpermissive temperature (Table 3), and DNA synthesis was induced.

DISCUSSION

Earlier studies have already indicated that infection of primary mouse kidney cells with polyoma virus results in an increase in cellular RNA, which follows the appearance of T-antigen (measured by immunofluorescence) and accompanies DNA replication (25). We have conducted a more detailed analysis of this process for two reasons. First, an increase in the amount of cellular RNA could be due either to increased rate of synthesis or to decreased rate of degradation. To decide between these two possibilities we measured RNA synthesis rates in isolated nuclei. Second, replicating cells generally have higher rates of RNA synthesis than resting ones. It remained to be shown, however, whether increased rate of RNA synthesis is merely a consequence of or a prerequisite for initiation of DNA synthesis in virus-infected mouse kidney cells. To answer this question, we performed time-course experiments starting early after virus infection.

Our results indicate that the increased amounts of cellular RNA are due (at least in part) to an increased rate of RNA synthesis (so far we cannot exclude that, in addition, RNA degradation is slowed down). This process occurs considerably earlier than the initiation of DNA replication and thus cannot simply be a consequence of replication. Together with a similarly early rise in ornithine decarboxylase activity (12), the increased rate of transcription is among the earliest responses of primary mouse kidney cells to infection with polyoma virus or SV40.

Analyses of RNA polymerases involved and of RNA species synthesized favor the idea that the activity of all three nuclear RNA polymerases is stimulated and that the result is a rather general increase in the rate of synthesis of all the RNA species that are already synthesized in uninfected cells. A similar conclusion was drawn by Salomon et al. (25) from an analysis of RNA synthesized in polyoma virus-infected mouse cells 24 h after infection, and more recently by Khandjian et al. (17a). All these experiments, of course, cannot exclude the possibility that a few new RNA species are produced in infected cells in addition to the general stimulation of RNA synthesis.

Our findings on the induction of cell division in resting mouse kidney cells by infection with polyoma virus or SV40 are strikingly similar to observations in other systems of growth stimulation, e.g., by hormones, growth factors, or serum (reviewed in references 21 and 24). For instance, there is good evidence that starved mouse fibroblasts respond to serum stimulation by a general increase in the rates of RNA and protein synthesis (16).

The mechanism of the increase in transcription rate is so far unknown. Our experiments indicate that it is primarily the rate of RNA

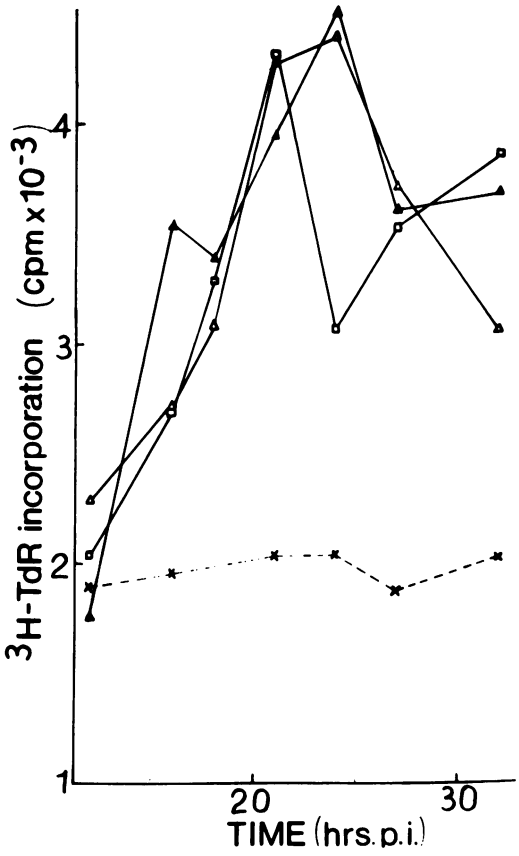

FIG. 6. *Induction of DNA synthesis in mouse kidney cells infected with SV40 deletion mutants dl884 and dl890. Confluent cultures of primary mouse kidney cells (in 60-mm petri dishes) were infected with 5 PFU of mutant or wild-type SV40 per cell at 37°C. DNA synthesis was measured by adding [^{3}H]thymidine (final activity, 1 μCi/ml) to the cultures at various times after infection. Incorporation of thymidine into DNA was determined as described in the text.* (×) *Mock-infected cells;* (□) *wild-type SV40;* (△) *dl884;* (▲) *dl890.*

chain elongation (rather than that of initiation) that is being measured in our assay system and that accounts for the elevated transcription activity of nuclei isolated from infected cells. This could be due to a modification of the RNA polymerases or to changes in chromatin structure. Although our chromatographic studies on RNA polymerases from uninfected and infected cells did not indicate gross changes in the charge of the enzymes, it cannot be excluded that modifications in one or more of the subunits of RNA polymerases are in fact responsible for the changes in transcription rate. This question can only be answered by a detailed study on RNA polymerases purified to near homogeneity from uninfected and infected mouse kidney cells, a project which meets with difficulties due to the very limited amounts of starting material available. As regards the second possibility, a change in chromatin structure, this could again be due to modification, in particular phosphorylation, of chromosomal proteins. We have carried out preliminary studies on the in vivo phosphorylation of nuclear proteins in uninfected and infected cells (unpublished data). So far these experiments do not provide evidence for specific changes in the phosphorylation pattern but indicate generally faster incorporation of phosphate into nuclear proteins in stimulated compared to resting cells.

The observation that the increase in transcription rate requires protein synthesis and cannot be observed when infection is carried out with virus that was previously UV-inactivated supports the idea that a virus function is involved in this reaction. Because T-antigens could not be observed at this early time in the infection cycle by immunofluorescence, we used a labeling technique to show that SV40 T-antigens are in fact already synthesized between 6 and 8 h after infection of mouse cells. Both large T- and small t-antigen could be demonstrated by specific immunoprecipitation; hence, either one or both of these proteins could be responsible for the early cellular reaction. Baserga and co-workers (1, 34) observed a stimulation of rRNA synthesis in isolated rat liver nuclei and nucleoli after addition of partially purified preparations of SV40 large T-antigen in vitro. These observations suggest an involvement of large T- rather than small t-antigen in our reaction. Since it is not clear, however, how far the stimulation of isolated nuclei in vitro corresponds to the reactions we measure in our time-course experiments, we used SV40 mutants to unequivocally answer this question. These experiments showed that the deletion mutants *dl*884 and *dl*890, which give rise to aberrant small t- but normal large T-antigens (5), behave like wild-type virus. Interestingly, the synthesis of both ribosomal and nonribosomal RNA is induced by these deletion mutants (just as by wild-type virus), as evidenced by results obtained with α-amanitin. Use of *tsA* mutants, which give rise to temperature-sensitive large T-antigen, gave a quite different result. In this case elevated transcription rates could only be induced by infection at 32°C, the permissive temperature, but not at 39°C, the nonpermissive temperature. We conclude from these data that SV40 large T-antigen is necessary for the early cellular reactions, whereas small t-antigen is not. In addition to the effect on transcription rates, those on the induction of DNA replication were followed in cells infected

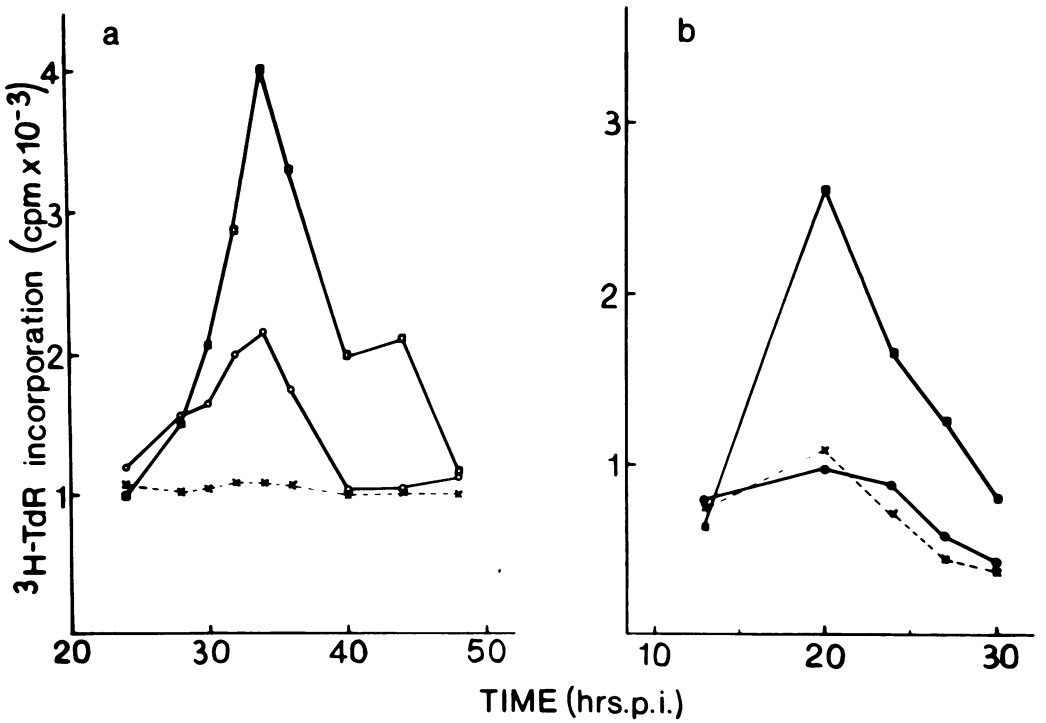

FIG. 7. *Induction of DNA synthesis in mouse kidney cells infected with SV40 mutant tsA209. Confluent cultures of primary mouse kidney cells (in 60-mm petri dishes) were infected with 5 PFU of mutant or wild-type SV40 per cell at 32°C (a) or at 39.5°C (b). DNA synthesis was measured by adding [^{3}H]thymidine (final activity, 1 μCi/ml) to the cultures at various times after infection. Incorporation of thymidine into DNA was determined as described in the text.* (×) *Mock-infected cells;* (□, ■) *wild-type SV40;* (○, ●) *tsA209. Open symbols, 32°C; full symbols, 39.5°C.*

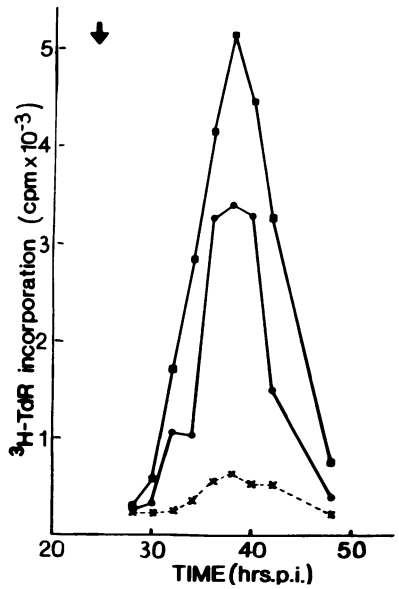

FIG. 8. *Induction of DNA synthesis in mouse kidney cells infected with SV40 mutant tsA209 after shift from the permissive to the nonpermissive temperature. Confluent cultures of primary mouse kidney cells (in 60-mm petri dishes) were infected with 5 PFU of mutant or wild-type SV40 per cell at 32°C. After 24 h incubation at 32°C, temperature was shifted to 39.5°C, and DNA synthesis was measured by adding [^{3}H]thymidine (final activity, 1 μCi/ml) to the cultures at various times after the shift. Incorporation of thymidine into DNA was determined as described in the text.* (×) *Mock-infected cells;* (■) *wild-type SV40;* (●) *tsA209. The arrow indicates the time of the shift from 32 to 39.5°C.*

with wild-type and mutant virus, and the results obtained in both types of assay were in full agreement. They suggested that the effect on transcription is a prerequisite for the induction of DNA replication, a conclusion supported by temperature-shift experiments. All these results, of course, do not prove that the induction of increased transcription rate is the only mitogenic activity of large T-antigen. It is quite possible that T-antigen is required in addition in later stages of the preparation for DNA replication. From the results of the temperature-shift experiments we would have to conclude, however, that these reactions involve functions of T-an-

tigen that are not impaired by the *tsA* mutation.

Setlow and co-workers (28) have recently carried out extensive studies on the effect of *ts*/deletion double mutants on the induction of DNA synthesis in growth-arrested CV1 cells. They showed that these mutants (including the one used in our study) stimulate the synthesis of host cell DNA at the nonpermissive temperature in serum-starved CV1 cells. We could not observe such a stimulation in our system. It should be pointed out, however, that confluent cultures of primary mouse kidney cells are physiologically arrested and can only be stimulated to divide by infection with a tumor virus. The requirements of these cells for the induction of DNA synthesis may thus differ, at least in part, from those of serum-starved cells. In fact, the results of Setlow et al. (28) on starved CV1 cells and our data on primary mouse kidney cells could be interpreted to indicate at least two functions of SV40 large T-antigen in the induction of cellular DNA replication, only one of which (the one not influenced by the *tsA* mutation) is required in serum-starved CV1 cells.

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