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Viral RNA Synthesis and Levels of DNA-Dependent RNA Polymerases During Replication of Adenovirus 2

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The rates of RNA synthesis in cultured human KB cells infected by adenovirus 2 were estimated by measuring the endogenous RNA polymerase activities in isolated nuclei. The fungal toxin α-amanitin was used to determine the relative and absolute levels of RNA synthesis by RNA polymerases I, II, and III in nuclei isolated during the course of infection. Whereas the level of endogenous RNA polymerase I activity in nuclei from infected cells remained constant relative to the level in nuclei from mock-infected cells, the endogenous RNA polymerase II and III activities each increased about 10-fold. These increases in endogenous RNA polymerase activities were accompanied by concomitant increases in the rates of synthesis in isolated nuclei of viral mRNA precursor, which was monitored by hybridization to viral DNA, and of viral 5.5S RNA, which was quantitated by electrophoretic analysis on polyacrylamide gels. The cellular RNA polymerase levels were measured with exogenous templates after solubilization and chromatographic resolution of the enzymes on DEAE-Sephadex, using procedures in which no losses of activity were apparent. In contrast to the endogenous RNA polymerase activities in isolated nuclei, the cellular levels of the solubilized class I, II, and III RNA polymerases remained constant throughout the course of the infection. Furthermore, no differences were detected in the chromatographic properties of the RNA polymerases obtained from infected or control mock-infected cells. These observations suggest that the increases in endogenous RNA polymerase activities in isolated nuclei are not due to variations in the cellular concentrations of the enzymes. Instead, it is likely that the increased endogenous enzyme activities result from either the large amounts of viral DNA template available as a consequence of viral replication or from functional modifications of the RNA polymerases or from a combination of these effects.

Expression of the adenovirus 2 genome during productive infection is a complex and highly regulated process (6, 9, 14, 23, 32, 36). The viral mRNA's synthesized before DNA replication (early infection) are encoded by a limited portion of the viral genome, whereas those present after replication (late infection) include transcripts of most of the genome. In addition to the viral mRNA's, a small, virus-coded 5.5S RNA species has been shown to be synthesized in large amounts during late infection (17, 19). Although the sequence of this RNA has been determined, its function is unknown (17-19). These temporal changes in viral gene expression during productive infection may reflect qualitative changes in transcription of the viral genome. Alternatively, the patttern of nuclear RNA processing and transport may control the formation of functional viral mRNA's. Several reports have demonstrated that nuclear RNA isolated from cultures either early or late in infection contains viral sequences not present in the cytoplasm (6, 14, 21, 23, 32, 37, 38). More recent studies have also identified symmetrical transcripts in early (S. Zimmer and H. J. Raskas, manuscript in preparation) and late nuclear RNAs (22, 23, 32).

Despite the uncertainty regarding the mechanism that controls the appearance of viral mRNA's, it is clear that there are dramatic increases in the rates of total RNA synthesis (host plus viral) as evidenced by studies of RNA synthesis in intact cells (7, 24) and in isolated nuclei (4, 39). Most of these changes represent increased viral gene transcription (9, 14, 23). In contrast, the rates of synthesis of host 4S RNAs (13, 27), 45S rRNA (13, 27), and heterogeneous

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nuclear RNA (HnRNA) (23) appear to be significantly inhibited late in infection, although detailed studies of these RNAs (especially HnRNA) have not been reported in adenovirus 2-infected cells.

The synthesis of virus-specific RNAs is mediated, at least in part, by RNA polymerases that cannot be distinguished from the cellular DNA dependent RNA polymerases (1, 25, 26, 39, 40, 41). Previous studies have suggested that all eukaryotes possess class I, II, and III RNA polymerases (reviewed in reference 28). These enzymes differ in catalytic and chromatographic properties (28), subunit composition (28, 34), and function. Thus, class I, II, and III RNA polymerases transcribe, respectively, the genes for rRNA (42), the genes for heterogeneous nuclear and presumably mRNA (28, 42, 43), and the tRNA and 5S RNA genes (40, 42). Similarly, in adenovirus-infected cells studies of RNA synthesis in isolated nuclei strongly suggest that RNA polymerase II transcribes DNA-encoding mRNA or mRNA precursors, that RNA polymerase III transcribes the gene encoding the viral 5.5S RNA (40, 41), and that RNA polymerase I does not transcribe any adenovirus genes (39–41).

The objectives of the present studies were to determine the relative contribution of each class of RNA polymerase to the increased rates of synthesis of total and virus-specific RNAs and to investigate the mechanisms responsible for the altered enzymatic activities. The endogenous activity of each class of RNA polymerase was quantitatively determined in nuclei isolated from cultured cells at different times after adenovirus infection, and the activities were related to the synthesis of virus-specific products. To analyze further the basis for the reported increases in transcriptional activities, cellular concentrations of the individual RNA polymerases were determined after solubilization and chromatographic resolutions.

MATERIALS AND METHODS

Cell culture and virus infection. Exponentially growing KB cell cultures were infected with adenovirus 2 at a multiplicity of 40 PFU/cell as described previously (5) and diluted to 3×10^5 cells/ml after a 1-h adsorption period.

Nuclear isolation and endogenous RNA polymerase assays. Nuclei were prepared from cells swollen in hypotonic buffer as previously described (40) and resuspended in buffer A (0.05 M Tris-hydrochloride, pH 7.9, 25% [vol/vol] glycerol, 5 mM $MgCl_2$, 0.1 mM EDTA, and 0.5 mM dithioerythreitol) at 3×10^7 nuclei/ml. Nuclei were assayed as described (40, 42) in the presence of the indicated concentration of ammonium sulfate and with 0.05 mM [^{3}H]UTP (Amersham Searle, 0.6 Ci/mmol). After incubation for 20 min at 37 C the reactions were pipetted onto DEAE-cellulose disks and processed as described (40, 42).

Hybridization and electrophoretic analysis of RNA synthesized in isolated nuclei. High-specific-activity RNA was extracted from isolated nuclei incubated as described above, except that the specific activity of the radioactive [^{3}H]UTP was increased to 10 to 49 Ci/mmol and the incubations were carried out for 10 min at 25 C. Reactions were terminated by DNase treatment at 0 C (40), followed by phenol-chloroform extraction in the presence of sodium dodecyl sulfate (20) and ethanol precipitation. RNA was analyzed either by hybridization to viral DNA immobilized on nitrocellulose membranes (5, 6) or by electrophoresis on 12% polyacrylamide gel slabs as described previously (40).

Solubilization of RNA polymerase activity. Adenovirus-infected or mock-infected cells were resuspended in buffer A at a concentration of 0.5×10^8 to 1×10^8 cells/ml and the RNA polymerases were solubilized by a modification of previously described methods (11, 31). Cells were lysed by the addition of 4 M ammonium sulfate (pH 7.9) to a final concentration of 0.3 M. The viscous solution was sonicated five to seven times for 10 s with a Branson S-75 Sonifier (ultramicroprobe, setting 1) at 0 to 4 C. The suspension (F1A) was subjected to centrifugation for 60 min at 55,000 rpm. The supernatant (F1B) was applied to a column of DEAE-cellulose (Whatman DE-52) equilibrated with buffer A containing 0.3 M ammonium sulfate. The extract was loaded at less than 1 mg of nucleic acid per ml of column bed volume. The sample was washed through the column with buffer A containing 0.3 M ammonium sulfate, and the unadsorbed protein fraction (F2) was collected and dialyzed against buffer B (buffer A without $MgCl_2$) until the ammonium sulfate concentration of the dialysate was 0.05 M (F3). The dialysate was then subjected to centrifugation for 60 min at 55,000 rpm. The supernatant (F4) was quick frozen in a dry ice-ethanol bath and was stored at −70 C until ion exchange chromatography. All centrifugations were performed at 0 to 4 C with a Spinco type 65 rotor.

DEAE-Sephadex chromatography. DEAE-Sephadex columns were prepared as described (11, 31) and equilibrated with buffer B containing 1 mg of bovine serum albumin per ml and 0.05 M ammonium sulfate. Fraction F4 was applied at less than 2 mg of protein per ml of bed volume. The columns were washed with one column volume of buffer B containing 1 mg of bovine serum albumin per ml and 0.05 M ammonium sulfate, and the RNA polymerase activities were then eluted with a linear, ammonium sulfate gradient (0.05 to 0.5 M) in buffer B containing 1 mg of bovine serum albumin per ml.

RNA polymerase assay. The assay conditions were as described (11, 31). Mouse plasmacytoma DNA and poly[d(AT)] (Miles) were used at final concentrations of 85 and 25 μg/ml, respectively. One unit of activity represents the incorporation of 1 pmol of UMP into RNA in 20 min at 37 C under standard assay conditions.

RESULTS

The first series of experiments to be described was designed to estimate the relative contribution of each of the major classes of cellular RNA polymerases to the increased rate of total RNA synthesis that occurs during viral infection and to establish whether changes in the intracellular activities of specific classes of RNA polymerase can be correlated with increased rates of synthesis of specific classes of viral RNA. Isolated nuclei offer several distinct advantages for these analyses of RNA synthesis. The RNA species synthesized in vitro resemble those found in vivo (discussed in references 40 and 42), and the rates of synthesis in vitro can be readily quantitated since nuclei are freely permeable to exogenous nucleotides of known specific activities. In addition, nuclei are freely permeable to the fungal toxin α-amanitin, which can be used to distinguish the relative activities and functions of the endogenous class I, II, and III RNA polymerases (31, 40, 42). RNA polymerase I is completely resistant to 200 μg of α-amanitin per ml, RNA polymerase II is completely inhibited by 1 μg of α-amanitin per ml, and RNA polymerase III is resistant to α-amanitin concentrations of 1 μg/ml but completely inhibited by 200 μg/ml. Thus the contribution of each class of RNA polymerase can be calculated from assays of endogenous nuclear RNA synthesis at α-amanitin concentrations of 0, 1, and 200 μg/ml.

Effect of salt concentration on the endogenous RNA polymerase activities in isolated nuclei. Because of the known effects of ionic strength upon in vitro transcription (3), it is important to consider this parameter for quantitative studies of RNA synthesis in isolated nuclei. As reported previously (41), the ionic strength optima of the endogenous RNA polymerase activities in isolated nuclei are distinct from those of the corresponding purified enzymes on exogenous templates. Furthermore, preliminary data (41) indicated that the ionic strength optima for the endogenous RNA polymerase activities differed in nuclei isolated from adenovirus-infected versus uninfected cells. Figure 1 shows a more detailed analysis of the effects of salt concentra-

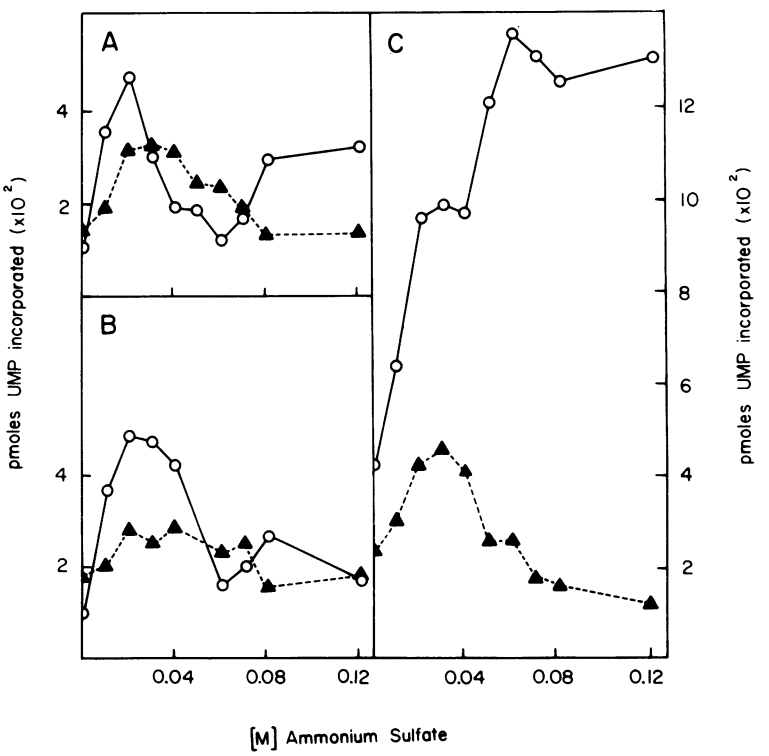

FIG. 1. *Effect of salt concentration on endogenous RNA synthesis in isolated nuclei. 2 x 10^6 nuclei isolated from mock-infected cells (A) or from cells harvested at 6 h (B) or at 14 h (C) after adenovirus infection were incubated as described in the text, in the presence and absence of 1 μg of α-amanitin per ml. The α-amanitin-resistant activity (▲) represents the combined endogenous RNA polymerase I and III activities, and the α-amanitin-sensitive activity (O) represents the endogenous RNA polymerase II activity (see text), normalized for 3 x 10^7 nuclei.*

tion of endogenous RNA polymerase activities in nuclei isolated from uninfected cells (panel A), from cells harvested early (6 h) after adenovirus infection (panel B), and from cells harvested late (14 h) in infection (panel C). In these experiments endogenous RNA polymerase I and III activities (resistant to low α-amanitin concentrations) were not distinguished. However, the optimum for these combined activities was the same (0.02 to 0.04 M ammonium sulfate) in nuclei isolated from uninfected cultures and from cultures harvested both early and late in infection. The salt optimum of the α-amanitin-sensitive RNA polymerase II was also similar (0.02 M ammonium sulfate) in nuclei from mock-infected cells or from cells harvested 6 h after infection (Fig. 1A and B). However, the optimum shifted to higher salt concentrations (0.06 M ammonium sulfate) by 14 h after infection (Fig. 1C).

Levels of endogenous RNA polymerase activities in isolated nuclei during the course of productive infection. The levels of endogenous RNA polymerase activities in isolated nuclei were measured at course of infection. These activities were measured at the optimal salt concentrations determined in the experiments described above (Fig. 1). Total endogenous RNA polymerase activity in isolated nuclei was measured at 0.04 M ammonium sulfate. As reported previously (4, 39), by 14 h after infection this activity increased approximately fivefold above the level found in nuclei from mock-infected cells (Fig. 2A). After 14 h, the rate of RNA synthesis in isolated nuclei began to decline.

The endogenous RNA polymerase I activity (Fig. 2C) was measured at 0.02 M ammonium sulfate in the presence of 200 μg of α-amanitin per ml. Relative to the level in nuclei from mock-infected cells, this activity decreased slightly or

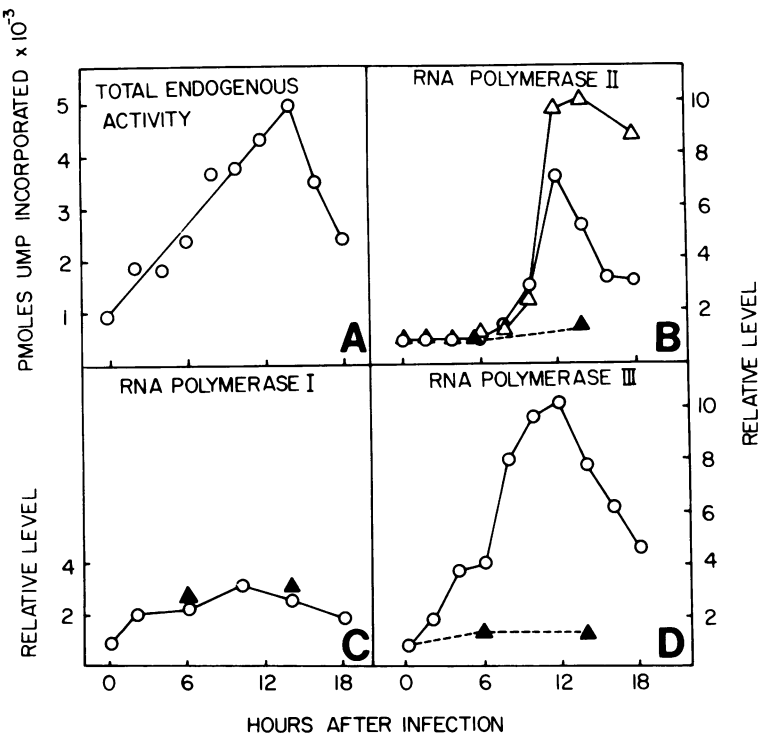

FIG. 2. *Changes in endogenous nuclear RNA polymerase activities during the course of adenovirus infection. (A) Total endogenous nuclear activity measured at 0.04 M ammonium sulfate* (○). *(B) Endogenous levels of RNA polymerase II activity in nuclei of mock-infected cells at 0.02 M ammonium sulfate* (▲) *or in nuclei of infected cells at 0.02 M ammonium sulfate* (○) *and at 0.06 M ammonium sulfate* (△). *(C) Level of endogenous RNA polymerase I activity in infected* (○) *and in mock-infected* (▲) *cells. (D) Endogenous levels of RNA polymerase III activity in nuclei from infected* (○) *and mock-infected* (▲) *cells assayed at 0.02 M ammonium sulfate. The individual RNA polymerase I, II, and III activities were distinguished with α-amanitin as described in the text. The levels indicated represent averages of duplicate determinations and have been normalized to 3 × 10^7 nuclei. The levels of UMP incorporated at 0 h in this particular experiment were, respectively, 181 pmol for RNA polymerase I, 360 (0.02 M ammonium sulfate) or 228 (0.06 M ammonium sulfate) pmol for RNA polymerase II, and 98 pmol for RNA polymerase III, per 3 × 10^7 nuclei in each case.*

stayed constant during the course of the infection. In different experiments this activity varied between 50 and 100% of the initial value, possibly a result of variations in viral multiplicity or synchrony of infection. In all instances, however, the endogenous RNA polymerase I activity represented only a small percentage (5 to 15%) of the total endogenous activity at late times after infection, in agreement with previous findings (40).

Analysis of the endogenous RNA polymerase II activity in isolated nuclei during the time course of infection is shown in Fig. 2B. In several different experiments the increases in endogenous RNA polymerase II activity relative to initial values varied between 5- and 10-fold when assayed at 0.02 M ammonium sulfate and between 8- and 12-fold at 0.06 M ammonium sulfate. At either salt concentration, endogenous RNA polymerase II activity peaked at 12 to 14 h after infection. Similarly, the level of endogenous RNA polymerase III activity (measured at 0.02 M ammonium sulfate) increased 6- to 10-fold relative to the initial values (Fig. 2D), with a maximum at approximately 12 to 14 h after infection. By 18 h after infection both the endogenous RNA polymerase II and III activities had decreased significantly (Fig. 2B and D). The increased endogenous RNA polymerase II and III activities in isolated nuclei were a result of the viral infection, for the endogenous RNA polymerase activities in nuclei from mock-infected cells increased less than twofold, an increase that can be attributed to the change in growth conditions of the cultured cells.

Synthesis of specific viral RNAs in isolated nuclei. Since the above measurements of RNA synthesis in isolated nuclei did not discriminate between transcription of host versus viral DNA sequences, viral transcripts were monitored directly. Two distinct classes of viral RNA transcripts were monitored since they are believed to be synthesized by distinct RNA polymerases (25, 26, 39, 40, 41). Most of the viral RNA synthesized in isolated nuclei is transcribed by an RNA polymerase II activity (25, 39, 40, 41). The endogenous RNA polymerase II transcripts, which appear to represent mRNA or mRNA precursors, were monitored directly by hybridization to viral DNA (Fig. 3). In this

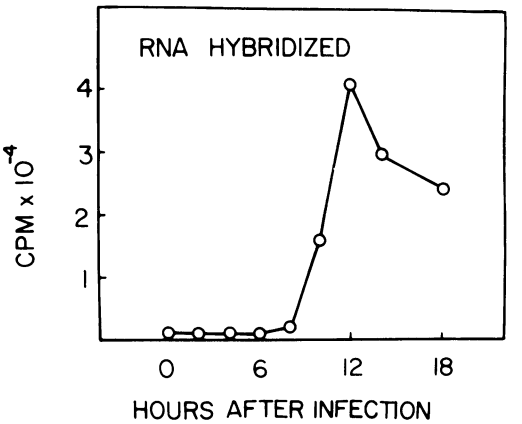

FIG. 3. *Synthesis of viral mRNA precursors in isolated nuclei during the course of infection. Nuclei were prepared from cells harvested at the indicated times after infection. At each time point the nuclei from 4 × 10⁶ cells were incubated with high-specific-activity [³H]UTP as described in the text. The total RNA present in each reaction was purified and hybridized to nitrocellulose filters containing 2.0 μg of viral DNA. Maximum hybridization in this experiment was 28% at 12 h after infection, although similar levels were observed at all later time points.*

experiment, the total RNA synthesized by a fixed number of nuclei, isolated at different times after infection, was hybridized with a constant amount of viral DNA. Although exhaustive hybridization conditions were not used, a 40-fold increase in the DNA/RNA ratios led to only a slight increase in the extent of hybridization. Thus, the maximum amounts of RNA hybridized at 14 h after infection represented 28 to 30% of the input, in agreement with the results obtained by others (39). As shown in Fig. 3, the rate of synthesis of viral mRNA or mRNA precursor increased to a maximum at 12 to 14 h after infection and then decreased, in good agreement with the observed increase and subsequent decrease in endogenous RNA polymerase II activity (see above paragraph and Fig. 2).

RNA polymerase III has been shown to synthesize several low-molecular-weight viral RNA species (40, 41; R. Weinmann, H. J. Raskas, and R. G. Roeder, manuscript in preparation) in isolated nuclei, in addition to the cellular

FIG. 4. *Synthesis of low-molecular-weight RNAs in nuclei isolated at different times during the course of infection. At each point nuclei from 3 × 10⁶ cells were incubated with high-specific-activity [³H]UTP as described in the text. RNA was extracted and run on 12% polyacrylamide gel slabs, which were sliced and counted. A small amount (500 counts/min) of electrophoretically purified ³²P-labeled 5.5S RNA synthesized in vivo was included as a marker (- - -). The peak co-migrating with the marker was thus identified as 5.5S RNA. Panels A through H show the synthesis, respectively, in nuclei isolated from cells harvested at 0, 4, 8, 10, 12, 14, 16, and 18 h after infection.*

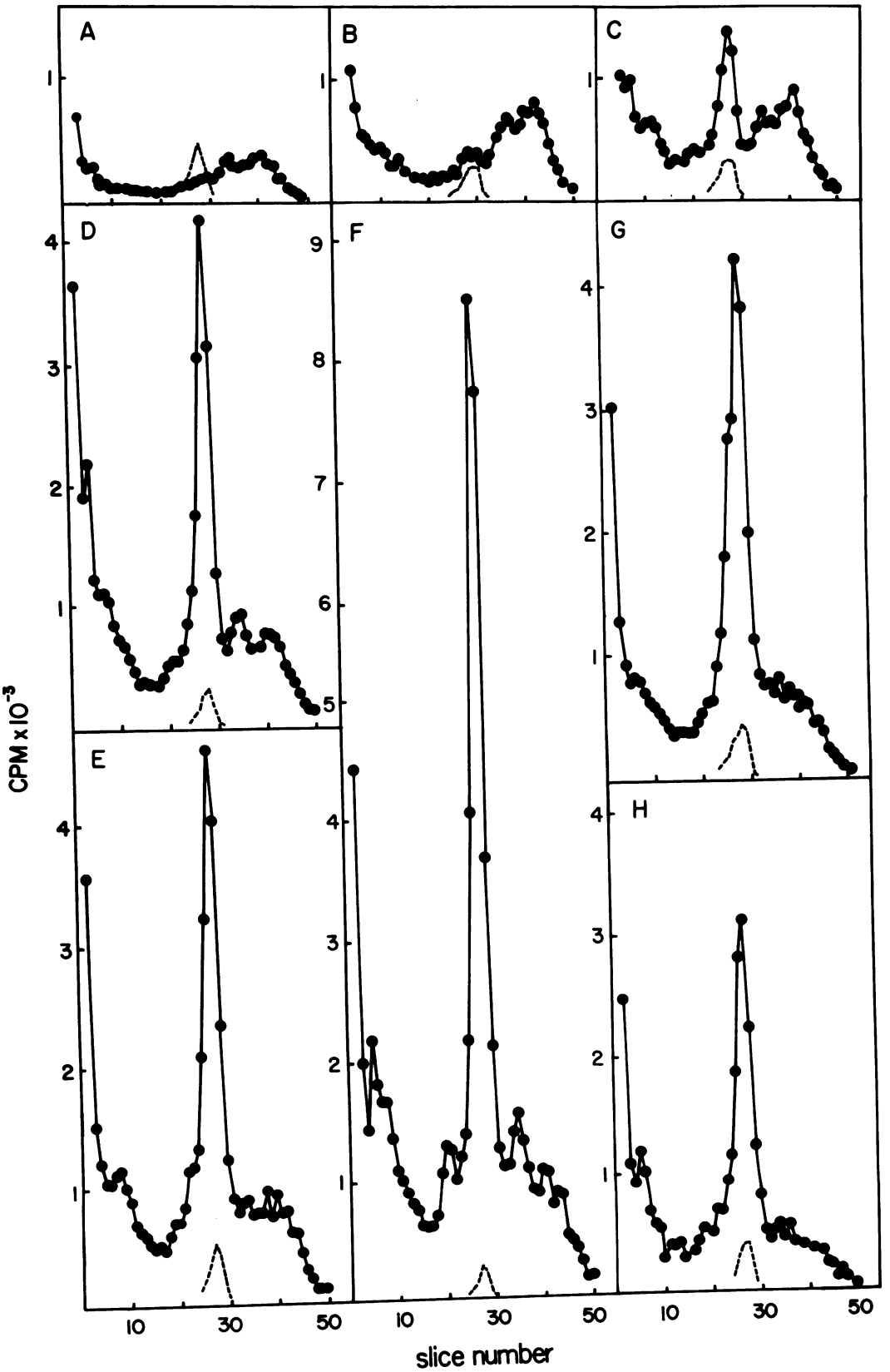

FIG. 4.

5S RNA and tRNAs (42). The viral 5.5S or virus-associated RNA represents the major low-molecular-weight RNA synthesized by isolated nuclei (18, 26, 40, 41) and has been shown to be coded by the viral genome (17). The identity of this in vitro transcript with the viral 5.5S RNA synthesized in vivo has been shown by co-migration on polyacrylamide gels (19, 26, 40, 41) and by hybridization competition (40). Figure 4 shows an electrophoretic analysis of low-molecular-weight RNA synthesized in nuclei isolated from cells at different times after adenovirus infection. 5.5S RNA synthesis can be detected as early as 4 to 8 h after infection, a time preceding or coincident with the onset of viral DNA synthesis (37). The result of these and similar experiments are summarized in Fig. 5. The maximum rate of 5.5S RNA synthesis occurred at 12 to 14 h after infection and declined to about half the maximal rate by 18 h after infection. These data correlate well with the above observations on endogenous RNA polymerase III activity, which showed a maximum at 14 h after infection and a subsequent decline (Fig. 2). These results indicate that in isolated nuclei increased rates of synthesis of specific viral RNAs can be correlated with increased levels of endogenous RNA polymerase activities.

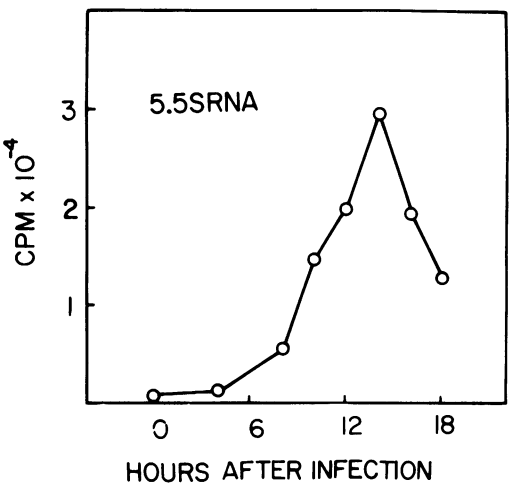

FIG. 5. *Synthesis of 5.5S RNA in isolated nuclei during the course of infection. The results of the experiment shown in Fig. 4 are summarized here. The [³H]RNA synthesized in isolated nuclei and co-migrating with the [³²P]5.5S RNA marker was integrated and plotted for each time point.*

Solubilization of RNA polymerases. To investigate the basis for the increased intracellular RNA polymerase activities during the course of infection, the enzymes were solubilized from infected cells, freed of endogenous templates, separated chromatographically, and assayed with exogenous templates. With heterologous nonspecific templates such as calf thymus DNA or synthetic poly[d(AT)], initiation and synthesis by the RNA polymerases appear to be largely nonspecific and the activities measured appear to reflect actual enzyme concentrations, independent of any regulatory modifications of the enzymes which may be manifest in vivo (29).

RNA polymerases were solubilized by using modifications (see Materials and Methods) of previously described procedures (23, 31). These changes were necessary because of the large increases in intracellular protein and nucleic acid content during infection (8). The major modification was the use of DEAE-cellulose to preferentially absorb most of the nucleic acids in the crude cellular extract, under ionic strength conditions (0.3 M ammonium sulfate)

TABLE 1. *Recovery of RNA polymerase activity after solubilization and DEAE-Sephadex chromatography*[a]

Fraction	RNA polymerase units/10⁸ cells					
	Mock infected			Infected		
	I + III	II	Recovery (%)	I + III	II	Recovery (%)
F1A	2,202	1,449	100	3,351	1,170	100
F1B	3,099	1,015	113	4,553	1,389	131
F2	2,436	2,072	123	2,542	2,240	106
F3	2,938	1,369	118	3,441	1,512	110
F4	4,932	764	156	4,522	2,812	162
DEAE-Sephadex	4,534	2,260	186	4,655	3,730	185

[a] The results shown are from one representative experiment. DEAE-Sephadex profiles for this experiment are shown in Fig. 5. Fractions F1A–F4, obtained as described in the text, were assayed at 0.06 M ammonium sulfate. Column fractions were assayed as described in Fig. 5. Units of RNA polymerase I + III activity in the crude fractions indicate activity resistant to 0.24 μg of α-amanitin/ml, whereas units of RNA polymerase II activity represent activity sensitive to 0.24 μg of α-amanitin/ml.

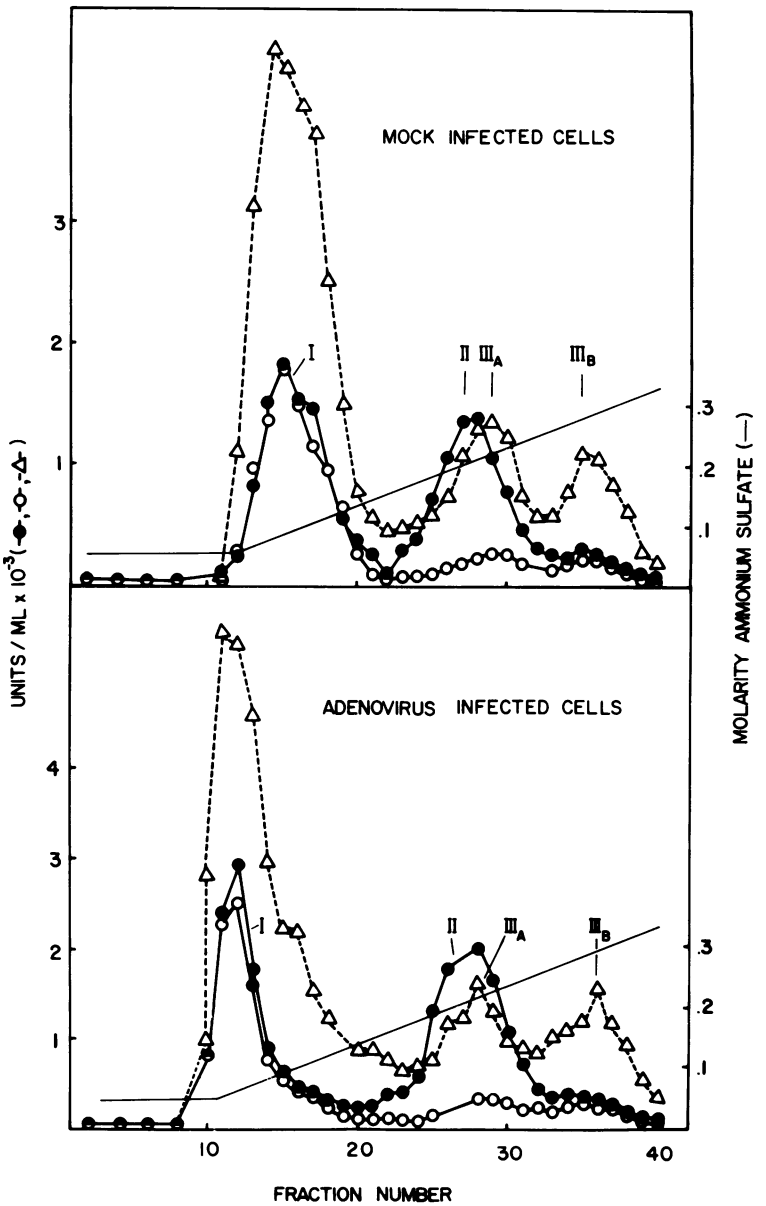

FIG. 6. *DEAE-Sephadex chromatography of RNA polymerases I, II, and III from uninfected and infected cells. RNA polymerase activity was solubilized and chromatographed as described in the text. Fraction F4 containing 25 mg of protein was obtained from 2 × 10⁸ mock-infected cells (upper panel) harvested at 14 h and was chromatographed on a 12-ml DEAE-Sephadex column. Fractions of 0.75 ml were collected after elution with a 30-ml linear gradient. Fraction F4 containing 41 mg protein was obtained from 2.8 × 10⁸ cells harvested at 14 h after infection (lower panel) and was chromatographed on a 20-ml DEAE-Sephadex column. Fractions of 1 ml were collected after elution with a 40-ml linear gradient. Activity in the fractions was measured at the salt concentration resulting from dilution of 10 μl of each fraction to a final assay volume of 25 μl. Assays were performed with mouse plasmacytoma DNA in the absence (●) or presence (○) of 0.24 μg of α-amanitin/ml or with poly[d(AT)] in the presence of 0.24 μg of α-amanitin/ml (△). (——) Ammonium sulfate concentration.*

where the RNA polymerases do not bind. The yields of activity through the various solubilization steps, detailed in Materials and Methods, are shown in Table 1 for two representative experiments in which mock-infected and infected cells were analyzed. No losses in activity were detected in either case, although these data do not rule out the possible

loss of an RNA polymerase whose activity is masked in crude extracts. In general, determinations of enzyme levels were found to be considerably more reproducible when determined after DEAE-Sephadex chromatography, probably due to the elimination of nonspecific inhibitors and the total dependence on exogenous templates. This may also account for the increased level of RNA polymerase II after chromatography on DEAE-Sephadex.

DEAE-Sephadex chromatography of solubilized RNA polymerases. The class I, II, and III DNA-dependent RNA polymerases present in solubilized extracts were resolved by chromatography on DEAE-Sephadex, as described previously (11, 31). A typical chromatographic profile of the solubilized enzymes from mock-infected and adenovirus-infected cells is shown

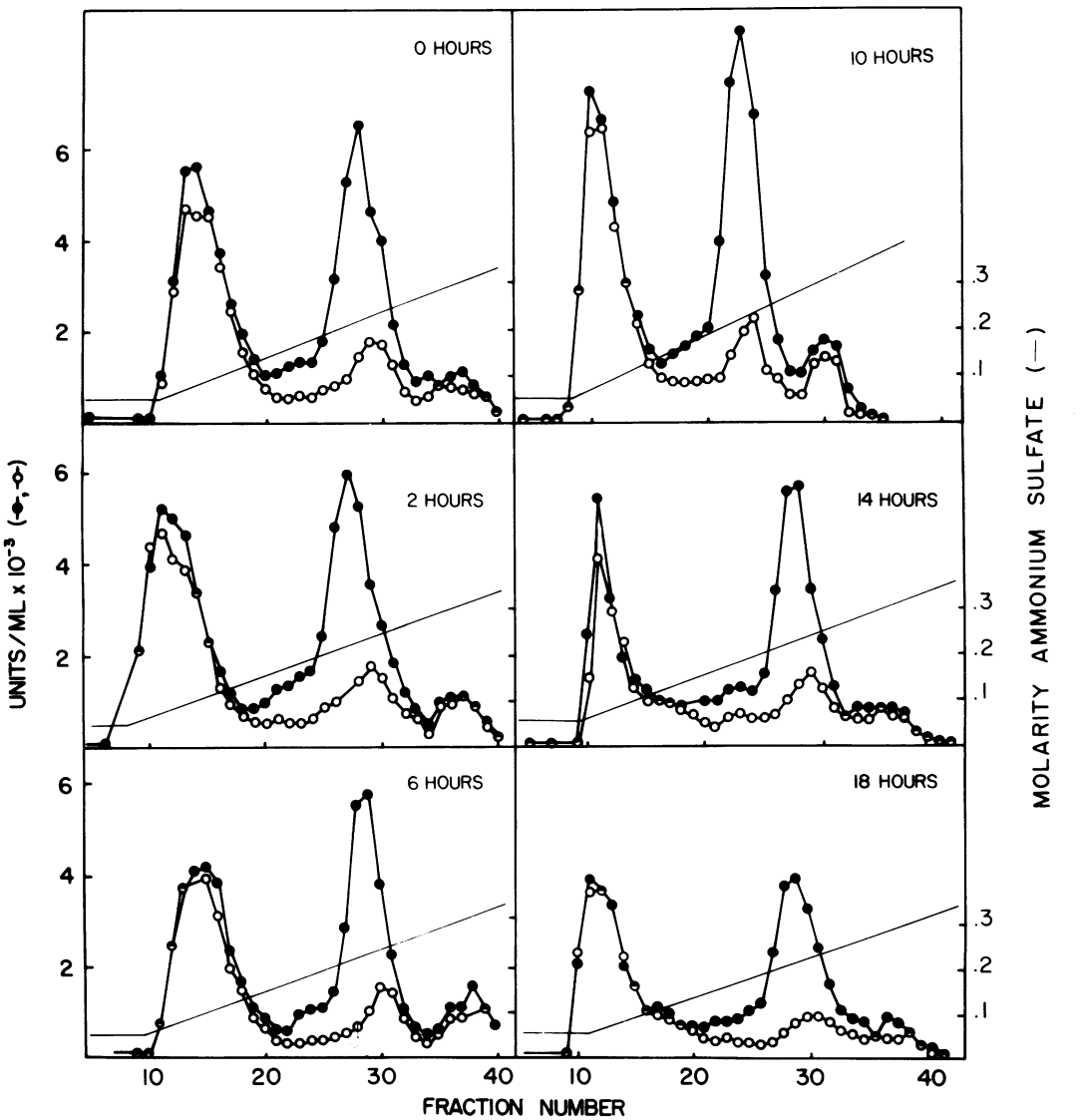

FIG. 7. *DEAE-Sephadex chromatography of RNA polymerases I, II, and III isolated from cells harvested at different times during the course of infection. RNA polymerase activity was solubilized as described in the text from 1.8 × 10⁸ cells harvested at the time points indicated. The F4 fractions obtained from cells at 0, 2, 6, 10, 14, and 18 h after infection contained 13.6, 15.2, 13, 18, 18.9, and 26.2 mg of protein, respectively, and were chromatographed on DEAE-Sephadex columns of 7, 8, 6.5, 9, 9.5, and 10.5 ml. The RNA polymerases were eluted, respectively, with linear gradients of 20, 20, 15, 25, 24, and 28 ml, and fractions of 0.5, 0.5, 0.42, 0.63, 0.63, and 0.71 ml were collected. Fractions were assayed as in Fig. 6 with poly[d(AT)] in the absence (●) or presence (○) of 0.24 μg of α-amanitin/ml. (——) Ammonium sulfate concentration.*

in Fig. 6. This profile is qualitatively similar to those obtained for enzymes solubilized from other mammalian tissues (11, 31). DNA-dependent RNA polymerase I eluted between 0.08 and 0.11 M ammonium sulfate and was completely resistant to high concentrations of α-amanitin. RNA polymerase II eluted between 0.20 and 0.23 M ammonium sulfate and was completely sensitive to 1 μg of α-amanitin per ml. When low concentrations of α-amanitin were used to inhibit RNA polymerase II in the gradient fractions, two forms of RNA polymerase III were also detected, as previously described in human lymphocytes and mouse myeloma (11, 31). One form eluted between 0.21 and 0.24 M ammonium sulfate (III_A), the other between 0.27 and 0.30 M ammonium sulfate (III_B). Both showed approximately fivefold greater activity with a poly[d(AT)] template relative to a DNA template (Fig. 6), and both were inhibited by high (200 μg/ml) but not by low (1 μg/ml) concentrations of α-amanitin (data not shown). Furthermore, when cellular levels of solubilized enzyme activities were compared between the infected and the mock-infected cells at 14 h after infection, no significant differences were detected for any of the three enzymes (Table 1). The difference in the RNA polymerase II levels detected in this particular experiment (Table 1) was not confirmed in other experiments.

Levels of solubilized RNA polymerases during the course of infection. Because of possible variations in the intracellular RNA polymerase activities during the course of infection, a more detailed analysis of the levels of solubilized RNA polymerases at different times after infection was performed. At each time point two aliquots of the infected cells were removed. One aliquot was used to measure the endogenous activities of the class I, II, and III RNA polymerases in isolated nuclei as described earlier. In this particular experiment the stimulation of total endogenous activity was sixfold, whereas the endogenous RNA polymerase II and III activities were stimulated eight- and sixfold, respectively (results not shown), in agreement with earlier results (Fig. 2). The second aliquot was subjected to enzyme solubilization and the extract was chromatographed on DEAE-Sephadex. To increase the sensitivity of the assay for the minor class III enzymes, only poly[d(AT)] was used as a template (reference 31 and above). Chromatographic profiles of RNA polymerases solubilized at different times of infection are shown in Fig. 7; the various profiles are indistinguishable. Thus it appears that neither α-amanitin sensitivities

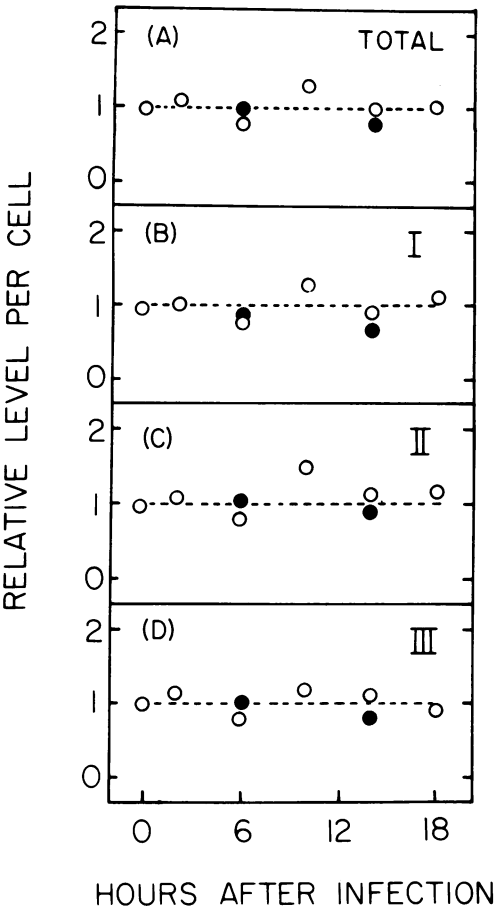

FIG. 8. *Relative levels of RNA polymerase activity during infection. The levels of enzyme activity of the infected cells are plotted relative to the levels of the enzyme at zero time. The data are calculated from those presented in Fig. 7. The level of solubilized RNA polymerase activity at zero time measured with poly[d(AT)] after DEAE-Sephadex chromatography was 16,834 units/10^6 cells. Of this total activity, 7,354 units corresponded to RNA polymerase I, 5,650 units to RNA polymerase II, and 3,830 units to RNA polymerase III.* (○) *Adenovirus-infected cells;* (●) *mock-infected cells.*

(40, 41) nor the chromatographic properties of the various RNA polymerases on DEAE-Sephadex (Fig. 6) are altered during the course of infection. Furthermore, when the solubilized RNA polymerase activity levels were quantitated and normalized, the level of total enzyme activity and the levels of the individual class I, II, and III enzymes remained constant on a per-cell basis throughout the entire course of adenovirus 2 infection (Fig. 8).

DISCUSSION

The infection of human KB cells with adenovirus 2 results in qualitative and quantitative

changes in RNA synthesis, especially during the transition from early to late gene expression, which coincides with the onset of viral DNA replication (9). During the course of infection, increased rates of viral RNA synthesis have been observed in both intact cells and isolated nuclei (see introduction). The present studies confirm and extend these observations. The rates of synthesis of two distinct classes of viral RNA and the levels of three distinct RNA polymerase activities have been monitored in nuclei isolated throughout the course of infection. The rates of synthesis of viral mRNA precursor and viral 5.5*S* RNA increase greatly during the infection, reaching maxima at about 12 to 14 h postinfection, and subsequently decline. Concomitant with these changes, both the endogenous RNA polymerase II and III activities increase about 10-fold, reaching maximum levels at 12 to 14 h, and then decline. The high efficiency of hybridization of RNA polymerase II transcripts to viral DNA (39; present data) supports the idea that RNA polymerase II products are predominantly viral. Similarly, hybridization competition studies support the idea that 5.5*S* RNA is the primary RNA polymerase III product although additional low-molecular-weight viral RNAs also appear to be synthesized by RNA polymerase III (Weinmann et al., manuscript in preparation). In agreement with the suggestion that viral genes are not transcribed by RNA polymerase I (40, 41), the activity of this enzyme is not increased during viral infection.

Of major interest are the mechanisms by which RNA polymerase activities and the rates of viral gene transcription are increased during viral infection. Possible mechanisms include (i) induction of virus-coded polymerases, (ii) increased concentrations or availability of cellular RNA polymerases, (iii) increased concentrations or availability of viral genes, (iv) modifications of the specificity or catalytic efficiency of specific RNA polymerases, or (v) combinations of these effects. The involvement of virus-coded polymerases seems improbable since the enzymes that transcribe viral genes have α-amanitin sensitivities identical to those of the cellular enzymes. Furthermore, the chromatographic properties of the RNA polymerases from infected and control cells are indistinguishable.

Increased concentrations of RNA polymerases have been correlated with increased rates of RNA synthesis or with changes in cell growth and division in a variety of cell types (11, 31, and references therein), indicating that RNA polymerase levels may in part determine the patterns of cellular gene expression. In contrast to these observations, the present studies strongly indicate that the cellular concentrations of the class I, II, and III RNA polymerases remain constant during the infection of cultured human cells by adenovirus 2, when increased rates of RNA synthesis are clearly evident, both in intact cells (4, 9, 14, 23, 24) and in isolated nuclei (4, 39; present studies). The increased rates of synthesis could be effected without changes in enzyme concentrations if the cellular enzymes are present in excess. Alternatively, if host gene transcription is impaired, the enzymes normally engaged in host transcription could become available for viral gene transcription. The possible inhibition of host transcription (13, 23, 27) argues in favor of the latter possibility. Furthermore, since enzyme concentrations fluctuate in response to the growth state of the cell in several normal cell types (above), it seems improbable that rapidly growing KB cells contain large pools of RNA polymerases not engaged in cellular gene transcription, which alone could account for the large increases in RNA synthesis observed upon viral infection.

Previous studies have shown that viral replication is required for the increased rates of viral gene transcription both in vivo (14, 15) and in isolated nuclei (26), suggesting that increased concentrations of viral genomes or replicating viral DNA are required for normal late transcription. It is possible that the viral DNA templates alone might suffice to sequester cellular RNA polymerases, either as a result of the high viral DNA concentration or as a result of high-affinity enzyme-binding sites in the viral DNA. Alternatively, other factors may also be necessary (in addition to increased viral DNA concentrations) to effect quantitative or qualitative changes in transcription. Thus viral infection might lead to alterations of the specificity or the activity of cellular RNA polymerases either as a result of enzyme subunit modifications or as a result of the accumulation of other factors that interact with the enzymes or with the templates. Although there is no direct evidence for either possibility, viral DNA sequences appear to be transcribed more efficiently than cellular genes if it is assumed that the RNA polymerases in KB cells are not in excess (see above). Both viral (10, 33, 39) and cellular (2, 12) genes appear to be transcribed from nucleoprotein complexes. However, it is possible that the respective viral and cellular nucleoprotein templates are sufficiently different in structure and composition to account for differences in the activities of the enzymes. The change in the

ionic strength optimum for the endogenous RNA polymerase II activity during the course of infection (Fig. 1) in fact suggests changes in enzyme template interactions (3).

The present data also permit consideration of the numbers of viral templates required to effect maximum rates of viral gene transcription. From the enzyme activity levels per cell (Table 1), the molecular weight of the purified enzymes (34), and the expected specific activities of the purified RNA polymerases (30, 34, 35; unpublished observations), it can be estimated that infected KB cells contain approximately 10^5 and 0.2×10^5 molecules per cell, respectively, of RNA polymerases II and III (cf. 30, 35). Assuming that active RNA polymerases are spaced in the DNA as described for other systems (16), it appears that considerably fewer than 10^4 viral genomes could accommodate all available enzyme molecules. Since as many as 10^6 viral DNA molecules may be present in infected cells (8), it is likely that only a small fraction of the viral genomes accumulated late in lytic infection are engaged in transcription at any one time. Since viral templates appear to be present in excess and since the class II and class III enzymes transcribe distinct regions of the adenovirus genome (40, 41), the possibility should also be considered that class II and class III enzymes do not simultaneously transcribe the same viral DNA molecule.

Previous studies have suggested that KB cells contain at least two distinct forms of RNA polymerase II which differ by a single subunit (cf. 30, 34, 35). The present studies also demonstrate that both infected and uninfected KB cells contain two distinct forms of RNA polymerase III, which in other mammalian cells differ in a single subunit (V. E. F. Sklar and R. G. Roeder, manuscript in preparation). Thus it is possible that individual molecular forms of the class II or class III enzymes are selectively modified or have distinct functions in the transcription of viral genes.

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