VIROLOGY 206, 100–107 (1995)

The Metabolism of Small Cellular RNA Species during Productive Subgroup C Adenovirus Infection

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Received January 19, 1994; accepted September 20, 1994

During the late phase of subgroup C adenovirus infection, export of cellular mRNA from the nucleus to the cytoplasm is inhibited. In one approach to investigate the mechanism whereby viral late mRNAs are selected for export, we have examined the metabolism of small cellular RNA species transcribed by all three RNA polymerases during the late phase of Ad5 infection. No changes in the quantities of [^{3}H]uridine-labeled 5S rRNA or tRNAs entering the cytoplasm were observed in infected cells. Adenovirus type 5 infection reduced the nuclear and cytoplasmic populations of the newly synthesized, snRNP-associated snRNAs U1, U2, U4, U5, and U6. Transcription of a representative snRNA, U1 RNA, was not inhibited, indicating that the post-transcriptional metabolism of snRNAs was perturbed during the late phase of infection. The increased cytoplasmic concentration of newly synthesized U1 RNA in Ad5- compared to mock-infected cells, and the greater reduction of the snRNP-associated compared to the total U1 RNA population, indicated that snRNP assembly in the cytoplasm was impaired. As adenovirus infection does not perturb export from the nucleus of small cellular mRNAs transcribed by RNA polymerases II and III, viral mRNA must be distinguished for selective export at a nuclear step upstream of translocation to the cytoplasm via nuclear pore complexes.

INTRODUCTION

Infection of permissive cells by subgroup C human adenoviruses is characterized by a temporally regulated program of viral gene expression that culminates in the production and assembly of large quantities of virus-specific macromolecules. Although viral gene expression is primarily controlled at the level of transcription (see Sharp, 1984; Berk, 1986; Flint, 1986; Shenk and Flint, 1991), subgroup C adenoviruses have evolved post-transcriptional mechanisms to subvert cellular biosynthetic systems to the production and utilization of virus-specific mRNAs: Viral mRNA species are selectively translated during the late phase of infection (see Flint, 1984; Schneider and Shenk, 1987; Mathews and Shenk, 1991) and from about 12 hr after infection, newly synthesized viral mRNAs preferentially accumulate in the cytoplasm (Beltz and Flint, 1979; see Flint, 1984). Transcription and processing of cellular pre-mRNAs continue during the late phase of infection, when newly synthesized cellular mRNAs fail to enter the cytoplasm (Beltz and Flint, 1979; Flint *et al.*, 1983; Babich *et al.*, 1983; Babiss *et al.*, 1985; Pilder *et al.*, 1986; Williams *et al.*, 1986), indicating that some aspect of the process by which mature mRNAs are exported from the nucleus to the cytoplasm must be perturbed. Selective export of newly synthesized viral mRNAs from the nucleus requires two viral early gene products, the E1B 55-kDa (Babiss *et al.*, 1985; Pilder, *et al.*, 1986; Williams, *et al.*, 1986) and the E4 ORF6 34-kDa (Halbert *et al.*, 1985; Weinberg and Ketner, 1986) proteins. These proteins interact with one another (Sarnow *et al.*, 1984) and the E1B 55-kDa–E4 34-kDa protein complex appears to induce the alterations in mRNA export characteristic of the late phase of adenovirus infection (Cutt *et al.*, 1987; Bridge and Ketner, 1990). Neither protein is intimately associated with nuclear pore complexes (NPCs) (Smiley *et al.*, 1990; Ornelles and Shenk, 1991), the cellular machines responsible for export of RNA from, and import of many proteins into, eukaryotic nuclei (see Goldfarb, 1989; Akey, 1992; Osborne and Silver, 1993). Rather, localization of the E1B 55-kDa–E4 34-kDa protein complex near infected cell inclusion bodies believed to be the nuclear sites of replication and transcription of adenoviral DNA (Ornelles and Shenk, 1991) has suggested that this protein complex might ensure that viral, late transcripts are synthesized and processed in nuclear microenvironments that permit, or facilitate, their subsequent export. Nevertheless, the mechanism responsible for selective export of viral, late mRNAs is not well understood.

In addition to mature mRNAs, a variety of other RNA species must be exported from the nucleus. Although pre-rRNA and rRNA species, like RNA polymerase II transcripts, spend a significant period in the nucleus, they

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0042-6822/95 $6.00

are synthesized by RNA polymerase I in the nucleolus, and subjected to distinctive processing and assembly reactions prior to their export to the cytoplasm (see Crouch, 1984; Hadjiolev, 1985). Small, cellular RNAs, by contrast, leave the nucleus rapidly and in some cases, notably abundant, small, nuclear RNAs (snRNAs), are largely processed in the cytoplasm (see Mattaj, 1988; Zieve and Sauterer, 1990). Irrespective of their sites of synthesis within the nucleus, the nature of the reactions by which they are processed or the proteins with which they assemble, these cellular RNAs are translocated from the nucleus to the cytoplasm via NPCs (see Maquat, 1991; Schröder *et al.*, 1987; Izaurralde and Mattaj, 1992). Export of each class of mature RNA is, furthermore, a facilitated and ATP-dependent process, and tRNA can compete with mRNA export (Dargemont and Kuhn, 1992), properties consistent with translocation via common machinery. Thus, in adenovirus-infected cells, modulation of mRNA export at the translocation step would be expected to interfere with export of other classes of cellular RNAs. We have, therefore, examined the metabolism of small cellular RNA species transcribed by RNA polymerases I, II, and III during productive adenovirus infection. In the course of these experiments, a defect in the nuclear accumulation of newly synthesized snRNAs was observed.

MATERIALS AND METHODS

Cells and virus

HeLa cells were maintained in suspension or monolayer cultures in minimal essential medium (SMEM or DMEM, respectively, Gibco) supplemented with 5% bovine serum (Gibco). Adenovirus type 5 was propagated and titered by plaque assay on HeLa cell monolayers as described (Flint *et al.*, 1975).

Preparation and analysis of labeled RNA

Uninfected HeLa cells, or Ad5-infected cells at various points in the infectious cycle, were labeled for 2 or 3 hr at 37° after concentration to $2-3 \times 10^6$ cells/ml in SMEM containing 0.04–0.3 mCi/ml [^{3}H]uridine (NEN-Dupont, 30–50 Ci/mmol). At the end of the labeling period, cells were harvested by centrifugation at 700 rpm for 3 min at 4° in a Beckman GPR centrifuge and washed in ice-cold phosphate-buffered saline. In some experiments, cytoplasmic extracts were prepared using 0.01 Tris–HCl, pH7.4, containing 0.10 *M* NaCl, 1.5 m*M* $MgCl_2$, and 0.5% (v/v) NP-40. In others, cells were fractionated into soluble, cytoskeletal, and nuclear fractions as described (Chatterjee and Flint, 1986). Cytoplasmic, soluble, or cytoskeletal fractions were adjusted to 1% (w/v) SDS and digested with 120 μg/ml proteinase K for 30 min at 37°. After organic extraction, RNA was precipitated with 2.5 vol of ethanol at −20°. To isolate nuclear RNA, nuclei were resuspended at 1.25×10^7 nuclei/ml in 0.01 *M* Tris–HCl, pH8.0, containing 0.01 *M* $MgCl_2$ and 0.01 *M* $CaCl_2$, and DNAse I (RNAase free, Promega) was then added to a final concentration of 25 units/ml. Samples were incubated at 37° for 30 min, adjusted to 1% (w/v) SDS, and deproteinized as described previously. The nucleic acids were ethanol precipitated, collected by centrifugation at 15,000 *g* for 15 min, air dried, and redigested with DNase I and proteinase K. In some experiments, the poly(A)-lacking cytoplasmic RNA population was prepared from total cytoplasmic RNA by chromatography on oligo(dT)-cellulose as described (Castiglia and Flint, 1983).

Preparation and immunoprecipitation of snRNPs

Uninfected or Ad5-infected HeLa cells were separated into cytoplasmic and nuclear fractions as described in the previous section. To extract nuclear snRNPs, nuclei were resuspended at approximately 10^7 nuclei/ml in 0.01 *M* Tris–HCl, pH 8.5, containing 0.1 *M* NaCl and 1 m*M* $MgCl_2$ and sonicated for a total of 40 sec while maintained in an ice bath. Samples were then centrifuged for 5 min at 4° in an Eppendorf microcentrifuge and the supernatant was taken as the nuclear extract. snRNPs were immunoprecipitated from the various fractions with Y-12 monoclonal antibody (Lerner *et al.*, 1981) IgG purified on protein A–Sepharose (Pharmacia). Immunoprecipitation, with quantities of purified Y-12 IgG and *Staphylococcus aureus* protein A determined by titration to be in excess, was as described previously (Smiley *et al.*, 1990). Immunoprecipitates were resuspended in 0.01 *M* Tris–HCl, pH 7.4, containing 0.1% (w/v) SDS and heated at 95° for 3 min. Samples were then adjusted to 0.3 *M* NaCl and the RNA was purified by organic extraction as described in the previous section. The RNA was precipitated with 2.5 vol of ethanol and stored at −80° under ethanol until analysis.

Electrophoresis of small RNA species

Labeled RNA was collected from ethanol suspensions by centrifugation, dried, and dissolved in 80% deionized formamide containing 1× TBE and 0.02% (w/v) each of bromphenol blue and xylene cyanol. Samples were incubated at 90° for 5 min, chilled rapidly, and applied to 10% (27:1) polyacrylamide gels containing 7 *M* urea cast in 1× TBE containing 1 mm EDTA. Electrophoresis was in the same buffer at 10–20 V/cm for 3–4 hr. Gels were then fixed by soaking in 10% (v/v) methanol and 10% (v/v) acetic acid and prepared for fluorography as described (Castiglia and Flint, 1983). In experiments to assess the steady-state levels of U1 snRNA, RNA purified from soluble, cytoskeletal, and nuclear fractions from mock- or Ad5-infected cells were electrophoretically transferred, following such electrophoresis, to 0.2-μm nylon mem-

branes at 50 V for 3 hr in 0.01 *M* sodium phosphate buffer, pH 6.5. The membranes were then air dried, baked *in vacuo* for 1 hr at 80°, and prehybridized for 1 hr at 65° under the conditions described by Church and Gilbert (1984). Hybridization was under the same conditions, for 12–16 hr, to about 0.2 μg DNA of the plasmid pU1, containing the human U1 snRNA ^{32}P-labeled by random primed synthesis (Feinberg and Vogelstein, 1984). Membranes were washed twice with 0.2× SSPE (1× SSPE is 0.18 *M* NaCl, 10 m*M* NaH_2PO_4, and 1 m*M* EDTA) containing 0.1% (w/v) SDS for 30 min at 50° and once with 0.2× SSPE at room temperature and exposed to Kodak X/AR film in the presence of an intensifying screen at −80°.

Hybridization of [^{3}H]-labeled RNA

Nuclear and cytoplasmic ^{3}H-labeled RNA was hybridized to membrane-bound U1 or pBR322 DNA, as described in the next section.

Run-on transcription in isolated nuclei

Nuclei were isolated from mock- or Ad5-infected HeLa cells as described (Flint *et al.,* 1984). Transcription reactions were as described (Flint *et al.,* 1984) and contained 0.2 mCi each of [α-^{32}P]UTP and [α-^{32}P]CTP (800 Ci/mmol, NEN-DuPont). After incubation at 37° for 45 min, nuclear RNA was purified as described in a previous section, except that two ethanol precipitations in the presence of 1 *M* ammonium acetate were included. The labeled RNA was then hybridized to nylon membranes on which pU1 DNA, L2 Ad2 DNA, human β-actin DNA, and pBR322 DNA had been immobilized. Each plasmid DNA was linearized by restriction endonuclease cleavage, heated at 100° for 10 min in 2× SSPE, and adsorbed to 0.2-μm nylon membranes using a Hybri-Slot Manifold (BRL). Membranes were prehybridized, hybridized to purified, labeled RNA, and washed as described in a previous section. Autoradiograms like that shown in Fig. 3 were quantitated by densitrometry of exposures in the linear range. Background values, represented by hybridization to pBR322 DNA, were subtracted, and the values (OD-cm^2) obtained then normalized to the number of cells in the mock-infected cell samples, determined by both direct counting of nuclei and measurement, by hybridization to slot blots, of the relative concentrations of HeLa cell DNA in a portion of each sample of nuclei.

RESULTS

Effects of subgroup C adenovirus infection on cytoplasmic accumulation of small cellular RNAs made by RNA polymerases I and III

To determine whether production of small, cellular RNA species were perturbed by adenovirus infection, we initially examined the accumulation of newly synthesized, low molecular mass RNAs in the cytoplasm of Ad5-infected cells. HeLa cells were infected with Ad5 and labeled with [^{3}H]uridine for 2 hr after increasing periods of infection, as described under Materials and Methods. As expected, adenovirus-encoded VA-RNA$_I$ was not detected in uninfected HeLa cells, but was synthesized from 4 hr after infection (Fig. 1, lanes 1 and 2). Moreover, as reported previously (Söderlund *et al.,* 1976; Mathews and Petterson, 1978), the rate of production of cytoplasmic VA-RNA$_I$ increased substantially between 8 and 12 hr after infection, following the onset of the late phase of infection. The appearance in the cytoplasm of newly synthesized 5.8S rRNA, a product of processing of the 45S pre-rRNA transcript synthesized by RNA polymerase I (see Crouch, 1984; Hadjiolev, 1985), was inhibited between 8 and 12 hr and reduced to undetectable levels by 18 hr after infection (Fig. 1, lanes 4–6). Analysis of the cytoplasmic levels of newly synthesized 28S and 18S rRNAs in the samples employed in the experiment shown

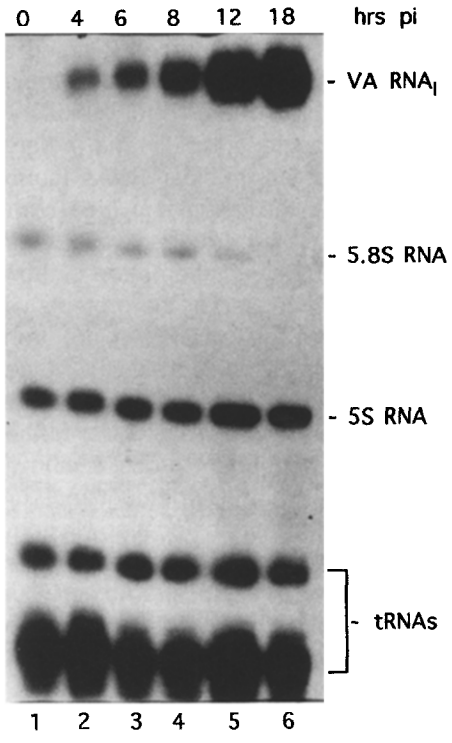

FIG. 1. Accumulation of newly synthesized, small RNA species in the cytoplasm of Ad5-infected cells. Mock- or Ad5-infected HeLa cells were labeled with [^{3}H]uridine for 2 hr after the periods of infection indicated, as described under Materials and Methods. Cytoplasmic, poly(A)-lacking RNA recovered from equal numbers of mock- or Ad5-infected cells was electrophoresed under denaturing conditions (see Materials and Methods). The small RNA species, indicated at the right, were identified by comparison of their relative mobilities with those reported previously under the same conditions of electrophoresis (Lerner *et al.,* 1981; Mathews and Petterson, 1978). Analysis of protein synthesis established that, in this experiment, inhibition of cellular protein synthesis was complete by 18–20 hr after infection (data not shown).

in Fig. 1 established that the cytoplasmic accumulation of this small rRNA decreased in parallel with inhibition of the cytoplasmic appearance of 28S rRNA (data not shown, but see Castiglia and Flint, 1983). By contrast, no significant change in the accumulation of newly synthesized tRNAs in the cytoplasm was observed as late as 18 hr after Ad5 infection (Fig. 1). Nor was the cytoplasmic accumulation of a second, RNA polymerase III transcript, 5S rRNA, inhibited (Fig. 1). Similarly, the cytoplasmic concentrations of newly synthesized 7S RNA species, also RNA polymerase III products (see Geiduschek and Tocchini-Valentini, 1988), were not reduced (data not shown). These observations indicate that adenovirus infection does not inhibit accumulation in the cytoplasm of abundant, small RNA species transcribed by RNA polymerase III.

Inhibition of snRNP-associated snRNA production in adenovirus type 5-infected cells

The lack of inhibition of appearance in the cytoplasm of small RNA species transcribed by RNA polymerase III in adenovirus-infected cells stands in sharp contrast to the inhibition of nuclear export of cellular mRNAs (see Introduction). We next investigated whether adenovirus infection perturbed the metabolism of small RNAs transcribed by RNA polymerase II, the abundant snRNAs. HeLa cells were infected with Ad5 and labeled with [^{3}H]uridine for 2-hr periods at various points in the infectious cycle, as in previous experiments. As all snRNAs could not be detected readily when total RNA populations were examined by electrophoresis and fluorography, they were first concentrated from nuclear and cytoplasmic extracts by immunoprecipitation with monoclonal antibody Y-12 (Lerner *et al.,* 1981). Small, nuclear RNA species, which were not recovered when immunoprecipitation was performed with M73 antibodies (Harlow *et al.,* 1985) against the adenovirus E1A protein (data not shown), were identified by their lengths and relative concentrations. Newly synthesized U1, U2, U4, U5, and U6 snRNAs could be readily detected using this procedure (Fig. 2A) and, as expected (see Busch *et al.,* 1982), were largely recovered in nuclear fractions. By 20 hr after Ad5 infection, the quantities of newly synthesized U1, U2, U4, U5, and U6 snRNAs recovered in nuclear RNPs from equal numbers of cells were significantly reduced, compared to mock-infected HeLa cells (Fig. 2A, lanes 3 and 4). When the total, ^{3}H-labeled RNA population was examined, no reductions in newly synthesized tRNAs or 5S rRNA were observed in Ad5-infected cell nuclear RNA preparations (Fig. 2B, lanes 3 and 4). The reduced quantities of nuclear snRNAs illustrated in Fig. 2A could be therefore attributed to a specific effect of Ad5 infection. Such inhibition was reproducibly observed during the late phase of infection and estimated to be three- to fivefold by 20 hr after infection (e.g., Fig. 2A).

The adenovirus-induced block to accumulation of newly synthesized snRNP-associated snRNAs in the nucleus is post-transcriptional

The results described in the previous section indicated that some step in the synthesis or maturation of cellular snRNAs is impaired during the late phase of adenovirus infection. To determine whether adenovirus infection inhibited snRNA transcription, we compared the rates of transcription of a representative snRNA, U1 RNA, in mock- and Ad5-infected cells using run-on transcription assays. As expected, the L2 region of the viral major late transcription unit was efficiently transcribed in late phase-infected, but not mock-infected, cells (Fig. 3). No reproducible decrease in the rate of U1 RNA transcription was observed in infected cell nuclei, compared to their uninfected cell counterparts; quantitation of data collected in several experiments like that whose results are shown in Fig. 3 indicated that the rate of U1 transcription during the late phase of Ad5 infection, relative to that observed in mock-infected cells, was 1.13 $\pm$ 0.36. The reduced accumulation of newly synthesized U1 snRNA in the nucleus cannot, therefore, be attributed to inhibition of their transcription. This result is not surprising for adenovirus infection does not induce a general inhibition of transcription by RNA polymerase III (see Introduction). Moreover, the inhibitory effects of infection illustrated in Fig. 2 would also require inhibition of RNA polymerase III, which is responsible for transcription of U6 genes (Kunkel *et al.*, 1986; Reddy *et al.,* 1987).

Adenovirus infection perturbs snRNP assembly

Inhibition of export of newly synthesized snRNAs from the nucleus would account for their reduced accumulation in nuclear snRNPs (Fig. 2A), for assembly of these ribonucleoproteins in the cytoplasm is required for their reentry into the nucleus (see Mattaj, 1988; Zieve and Sauterer, 1990). A specific block of export of snRNAs from the nucleus would result in inhibition of appearance of newly synthesized snRNAs in the cytoplasm, but no change in the rates at which they were processed, assembled, or reimported to the nucleus. In such a circumstance, infected cell cytoplasmic fractions would be selectively depleted of newly synthesized snRNAs. To investigate whether export of newly synthesized snRNAs were blocked following adenovirus infection, we therefore compared the distribution of [^{3}H]uridine-labeled RNA species between nuclear and cytoplasmic fractions in mock- and Ad5-infected cells. When newly synthesized, nuclear, and cytoplasmic snRNAs were examined following Y-12 antibody immunoprecipitation, reduced accumulation in the cytoplasm was observed during the late phase of infection (Fig. 2A, lanes 1 and 2), but no selective depletion of the cytoplasmic population was evident (Fig. 2A).

To examine the distribution of newly synthesized

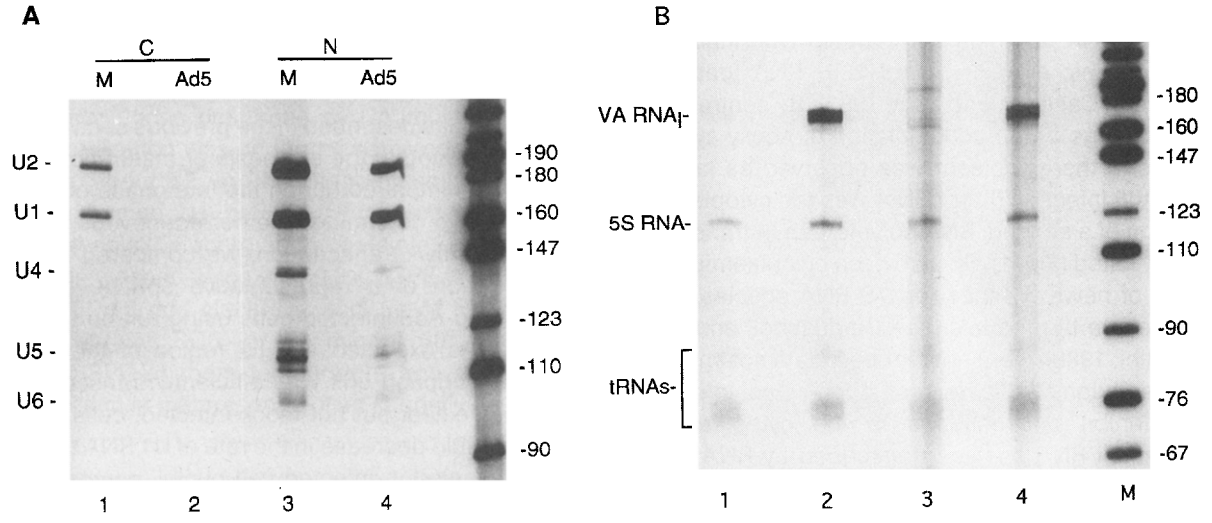

FIG. 2. Accumulation of newly synthesized snRNP-associated snRNAs in the nucleus and cytoplasm of adenovirus-infected cells. Cytoplasmic (lanes 1 and 2) or nuclear (lanes 3 and 4) extracts prepared from mock- (lanes 1 and 3) or Ad5- (lanes 2 and 4) infected HeLa cells labeled with [^{3}H]uridine for 2 hr were prepared as described under Materials and Methods. (A) The snRNAs recovered after immunoprecipitation of extracts made from equal numbers of cells with excess, purified Y-12 IgG were electrophoresed under denaturing conditions and detected by fluorography as described under Materials and Methods. (B) Total RNA purified from portions of each sample corresponding to equal numbers of cells was analyzed in the same way. Glyoxalated, end-labeled *Hpa*II fragments of pBR322 DNA were run in the lanes marked M in each panel. The identities of the small RNA species are indicated at the left of each panel.

snRNA more quantitatively, and to circumvent the requirement for assembly of these RNAs into snRNP, the concentrations of a representative snRNA, U1 RNA, were measured directly. Nuclear and cytoplasmic RNA populations were prepared from mock- and Ad5-infected cells that had been labeled with [^{3}H]uridine for 2 hr and hybridized to saturating quantities of the human U1 gene, as described under Materials and Methods. The results of such experiments are illustrated in Fig. 4A. Following Ad5 infection, the quantities of total, newly synthesized U1 snRNA recovered in nuclear and cytoplasmic fractions were significantly reduced. As transcription of U1 snRNA was not inhibited (Fig. 3), such reductions indicate that newly synthesized snRNA must be turned over more rapidly in Ad5-infected cells. The proportion of newly synthesized U1 snRNA that was recovered in the cytoplasm increased by a factor of 3 to 4 in Ad5-infected cells (C:N ratios in Fig. 4A). This increase in the fraction of the newly synthesized U1 snRNA population present in the cytoplasm was not a trivial consequence of increased extraction of snRNAs or snRNPs from infected-cell nuclei; as illustrated in Fig. 4B, no increase in the fraction of the steady state of U1 snRNA population recovered in cytoplasmic (that is S plus C in Fig. 4B) fractions was observed in cells harvested during the late phase of infection compared to mock-infected cells. The results shown in Fig. 4A therefore indicate that the proportion of newly synthesized snRNA recovered in the cytoplasm was specifically increased, rather than decreased (as predicted for an export block) following infection. This alteration suggests that snRNP import into the nucleus was directly or indirectly impaired during the late phase of infection.

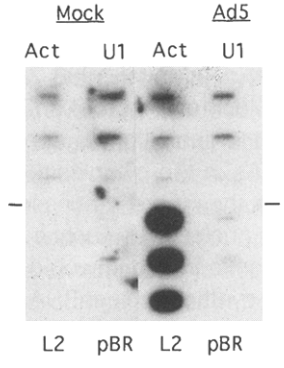

FIG. 3. Transcription of U1 RNA is not inhibited in adenovirus-infected HeLa cells. Nuclei isolated from mock- (M) or Ad5- (Ad5) infected HeLa cells harvested 16 hr after infection were used in run-on transcription reactions under the conditions described under Materials and Methods. The labeled RNA was purified and hybridized to filters carrying immobilized pBR322, Ad2 L2, β-actin, and U1 DNA, as indicated. Each filter carried three slots loaded with each of the four DNAs in decreasing quantities, namely, from top to bottom for each of the DNA samples, 4.5, 1.5, and 0.5 μg β-actin-containing plasmid, 3.0, 1.0, and 0.3 μg pU1 DNA, 4.5, 1.5, and 0.5 μg L2-containing plasmid, and 4.5, 1.5, and 0.5 μg pBR322 DNA. An autoradiogram of such a filter is shown.

As the accumulation of newly synthesized snRNAs in the nucleus depends on their interaction with snRNP proteins in the cytoplasm (see Mattaj, 1988; Zieve and Sauterer, 1990), we determined whether Ad5 infection altered the synthesis or accumulation of these proteins. Although synthesis of Sm proteins was inhibited, in paral-

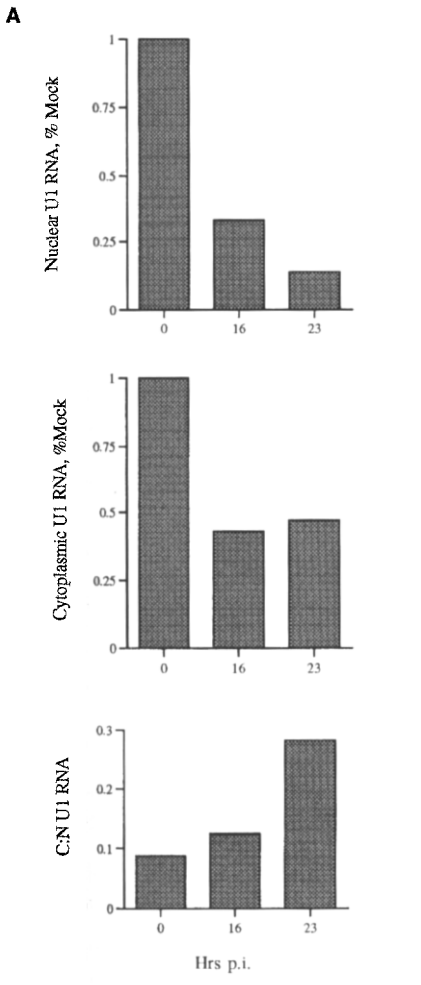

FIG. 4. Relative concentrations of newly synthesized U1 RNA in the nucleus and cytoplasm of adenovirus-infected cells. (A) The concentrations of total, newly synthesized, nuclear and cytoplasmic U1 RNA were measured by hybridization of ^{3}H-labeled RNA purified from nuclear and cytoplasmic fraction to excess, filter-bound U1 DNA, as described under Materials and Methods. RNA purified from an equal number of mock- and Ad5-infected cells, labeled at the times after infection indicated, was hybridized to duplicate filters. The values obtained are expressed relative to those of the mock-infected cell fractions and are the means of two independent experiments. The mock-infected values set at 1.0 in the two experiments were 8638 and 7112 cpm for nuclear RNA and 879 and 408 cpm for cytoplasmic RNA. The ratio of U1 RNA recovered in the cytoplasmic and nuclear fractions from each sample is shown in the panel designated C:N. (B) The steady-state concentrations of U1 snRNA in the soluble (S), cytoskeletal (C), and nuclear (N) fractions were examined in mock- and Ad5-infected (Ad5) cells by blotting, as described under Materials and Methods.

lel with synthesis of other cellular modest, only modest (less than twofold) decreases in the large cytoplasmic pool of Sm proteins were observed by immunoblotting (data not shown).

DISCUSSION

The results described here establish that one or more post-transcriptional steps in the pathway by which nuclear snRNPs are produced are perturbed during the late phase of adenovirus infection; significantly reduced quantities of newly synthesized snRNAs were present in infected cell nuclei (Figs. 2 and 4) at a time when no inhibition of transcription of a representative snRNA, U1 RNA, was observed (Fig. 3). The disruption of snRNA metabolism in adenovirus-infected cells can, however, be distinguished from the inhibition of production of cytoplasmic, cellular mRNA species. The partial reduction in the quantities of newly synthesized snRNA detected in the cytoplasm as late as 20–23 hr after infection (Figs. 2 and 4) strands in sharp contrast to the complete inhibition of cytoplasmic appearance of newly synthesized cellular mRNAs from as early as 12 hr after infection (Beltz and Flint, 1979; Castiglia and Flint, 1983; Babich *et al.*, 1983; Pilder *et al.*, 1986; Williams *et al.*, 1986). Furthermore, the increased proportion of newly synthesized U1 snRNA recovered in the cytoplasm of infected cells (Fig. 4) is inconsistent with a specific export block. Rather, this property indicates that newly synthesized snRNA species fail to reenter the nucleus efficiently in Ad5-infected cells. The greater decrease in the concentration of newly synthesized snRNA in the Sm-associated than in total cytoplasmic populations (Figs. 2A and 4) and the similar reductions in the nuclear and cytoplasmic populations of snRNP-associated, newly synthesized snRNAs (Fig. 2A) indicate that snRNPs do not assemble efficiently in the cytoplasm of infected cells. The increased turnover of newly synthesized snRNA observed in infected cells (see Results) seems likely to account for the reduction in quantities of snRNPs containing newly synthesized snRNA observed in infected cells. However, we cannot exclude the possibility that inhibition of Sm protein synthesis also contributes. Be that as it may, the disruption of production of abundant snRNPs reported here is unlikely to impede adenoviral gene expression during the late phase of infection, for no changes in the large, steady-state snRNA population (e.g., Fig. 4B) could be detected.

One consequence of infection of permissive cells by human subgroup C adenoviruses is severe inhibition of export of cellular mRNA species from the nucleus (see Introduction). Data presented here establish that small cellular RNA species, transcribed by RNA polymerase II or III are, by contrast, exempt from adenovirus-induced export inhibition; the quantities of newly synthesized 5S

rRNA or tRNAs accumulating in the cytoplasm were not reduced during the late phase of infection (Fig. 1), nor, as discussed in the previous paragraph, was any evidence obtained for inhibition of export of newly synthesized snRNAs. It is well established that small RNA species leave the nucleus via nuclear pore complexes (Dworetzky and Feldherr, 1988), the sites of import and export of cytoplasmic proteins and nuclear mRNPs, respectively (see Goldfarb, 1989; Akey, 1992). The continued export of some, but not other, cellular RNAs during the late phase of adenovirus infection cannot, therefore, be attributed to their exit from the nucleus by separate systems. Rather, the inhibition of export of only a subset of the cellular RNAs that are translocated via NPCs strongly suggests that NPC function and specificity are not directly modulated in adenovirus-infected cells. In this context, it is important to keep in mind that the definition of "export inhibition" is strictly an operational one, which includes all nonprocessing steps upon which exit of mature mRNAs from the nucleus might depend. These results therefore suggest that adenovirus infection alters the specificity of a reaction, upstream of NPC translocation, that determines efficient export of mRNA from the nucleus. As selective export of viral mRNA depends on the E1B 55-kDa and E4 34-kDa proteins (see Introduction), we propose that these viral early proteins alter the specificity of this, as yet unknown, reaction.

Ribosomal 5.8S rRNA exhibited exceptional behavior; its appearance in the cytoplasm was substantially inhibited during the late phase of infection (Fig. 1). Such inhibition does not appear to be a secondary consequence of the specific inhibition of RNAp I transcription that occurs by 19 hr after adenovirus infection (Learned *et al.,* 1983), for impaired entry of 5.8S rRNA into the cytoplasm was observed as early as 12 hr pi (Fig. 1). Such inhibition may be related to the selective inhibition of appearance in the cytoplasm of 28S rRNA that occurs during the first few hours of the late phase of infection (Castiglia and Flint, 1983); as both 28S rRNA and 5.8S rRNA are components of the 60S ribosomal subunit (see Hadjiolev, 1985), it is possible that the assembly or export of this ribonucleoprotein is disrupted in adenovirus-infected cells. However, neither the mechanism that might be responsible for a specific effect of adenovirus infection on rRNAs found in the larger ribosomal subunit nor its relationship to the E1B 55-kDa–E4 34-kDa protein complex-dependent inhibition of export of cellular mRNAs have yet been elucidated.

ACKNOWLEDGMENTS

We thank Joan Steitz and Alan Weiner for gifts of Y12 hybridoma cells and pU1 DNA, respectively, Ruchi Mathur for expert technical assistance and Norma Caputo for preparation of the manuscript. This work was supported by a grant to S.J.F. from the National Institutes of Health.

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