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Transcription of the Genome of Adenovirus Type 12

I. Viral mRNA in Abortively Infected and Transformed Cells

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In baby hamster kidney (BHK-21) cells abortively infected with adenovirus type 12, polysome-associated, virus-specific RNA could be detected starting 5 to 7 h after infection. The amount of this RNA reached a maximum between 10 to 12 h after infection and continued to be synthesized at a reduced level until late in infection (48 to 50 h). In BHK-21 cells transformed by adenovirus type 12 (HB cells), 0.26% of the polysome-associated mRNA was virus specific. The size of the virus-specific mRNA isolated from polysomes of BHK-21 cells abortively infected with, or transformed by, adenovirus type 12 was determined by electrophoresis in polyacrylamide gels in 98% formamide, i.e., under conditions which eliminated secondary structure or aggregation of RNA. In abortively infected hamster cells viral mRNA size classes of molecular weights 0.9×10^6 and 0.65×10^6 to 0.67×10^6 were predominant. A minor fraction of 1.5×10^6 daltons was consistently found and increased with time after infection. Late after infection (24 to 26 h), viral mRNA of 1.9×10^6 daltons was also observed. The size distribution of adenovirus type 12-specific mRNA from transformed hamster cells (HB line) was very similar to that in abortively infected cells, except that the relative amount of the viral mRNA fraction of 1.5×10^6 daltons was much higher. It is uncertain whether the viral mRNA of high-molecular-weight represents mixed transcripts derived from integrated viral genomes and adjacent host genes.

Baby hamster kidney (BHK-21) cells infected with adenovirus type 12 (Ad12) represent a model system of abortive virus infection which leads to malignant transformation in a small proportion of the infected cells (27). In nonproductive infection BHK-21 cells with Ad12 (4, 24, 25), the block in virus production is located in an early function, since viral DNA replication cannot be detected (4, 5, 6, 21), and synthesis of viral capsid proteins is not apparent (20). Starting at approximately 8 h after infection, the virus-specific T-antigen is synthesized. The time of onset of T-antigen synthesis depends on the actual multiplicity of infection. At 24 h after infection with high multiplicities, 100% of the cells contain T-antigen (24). The block for Ad12 replication does not seem to be due to a diffusible repressor-like function, since upon infection of heterokaryons of BHK-21 cells and permissive HEp2 cells with Ad12, viral antigens and viral DNA can be detected in both types of nuclei (31).

In cells arrested in G1 phase by serum depletion, cellular DNA synthesis is elicited starting at 12 h after infection with Ad12, and reaches a maximum at 21 to 24 h after infection (22). In growing BHK-21 cells, cellular DNA synthesis continues after infection with Ad12 at an increased rate (4). The cellular DNA synthesized starting 24 h after infection is cleaved to, or synthesized in, fragments of 5×10^6 to 10×10^6 daltons (4). Beginning 10 to 12 h after infection, fragments of the parental viral DNA are observed which sediment at a rate of 18*S* (2).

The DNA of Ad12 becomes integrated by covalent linkage, probably in the form of fragments, into the genome of BHK-21 cells (3, 5). This integration is first detectable at 6 h after infection and is not dependent on DNA replication, i.e., the viral genome integrates even if DNA replication is inhibited >96% by cytosine arabinoside (5). Inhibition of protein synthesis does not affect integration to a great extent (5). This finding suggests that the function(s) responsible for integration preexists in the cell or in the virion.

Transcription of the DNA of Ad12 in BHK-21 cells was investigated by Raška et al. (22) and by Raška and Strohl (21). Virus-specific RNA was found by these authors between 10 and 18

h postinfection. The size of this RNA was estimated to be approximately 18S (22). From the results of experiments using the technique of competition hybridization, it was concluded that the exclusively early RNA is transcribed in this system (21). About 60% of the RNA sequences transcribed early in the lytic system (21) are present in Ad12-infected BHK-21 cells. These sequences correspond to those which are transcribed exclusively early but not late in the productive infection (11, 21). In cells productively infected with adenovirus type 2, the early virus-specific RNA represents 8 to 45% of the viral genome (11, 30), corresponding to RNA of molecular weight of 0.9×10^6 to 5.0×10^6.

A review of the events occurring in Ad12-infected hamster cells was published recently by Strohl (26).

In the present report, the kinetics of appearance of the polysome-associated, Ad12-specific mRNA in BHK-21 cells is described. The size of the Ad12 mRNA was determined both in abortively infected BHK-21 cells and in the Ad12-transformed BHK-21 cell line, the HB cells. The main size class of viral mRNA both in abortively infected BHK-21 cells and in HB cells had a molecular weight of 9.0×10^5, but size classes of 1.5×10^6, 6.5×10^5, and 3.0×10^5 to 4.0×10^5 were also apparent. The relative amount of Ad12-specific mRNA in the size-class of 1.5×10^6 daltons increased with time after infection, and reached a maximum in the transformed HB cells.

MATERIALS AND METHODS

Cells. KB (CCL17) and BHK-21 (CCL10) cells were obtained from the American Type Culture Collection. BHK-21 cells were also obtained as a gift from P. Faulkner. Human embryonic kidney (HEK) cells used for plaque assays were purchased from Flow Laboratories. HB cells, a line of BHK-21 cells transformed by Ad12, were isolated by W. Strohl et al. of Rutgers University and were obtained from P. Choppin of Rockefeller University, New York.

Virus. Seed preparations of human Ad12 and adenovirus type 2 were a gift of W. Rowe, National Institutes of Health, Bethesda, Md.

Media and sera. The media used in our experiments were Eagle medium (10), the modification of Eagle medium for suspension cultures, and the Dulbecco modification of Eagle medium (1). Calf serum and fetal calf serum were purchased from Flow Laboratories, and tryptose phosphate broth was from Difco Laboratories.

Solutions. PBS, phosphate-buffered saline (9), and PBS-d, the same solution without Ca^{2+} and Mg^{2+}, were used. TE was 0.01 M Tris-hydrochloride (pH 7.5) and 0.001 M EDTA. Isotonic buffer for cell fractionation contained 0.15 M NaCl, 0.0015 M $MgCl_2$, and 0.01 M Tris-hydrochloride (pH 8.5). The sucrose solutions for polysome isolation were prepared as follows. To destroy ribonuclease contaminations, 10 and 50% solutions of sucrose in distilled water were incubated overnight with 0.1% diethylpyrocarbonate at 37 C and subsequently for 30 min at 100 C to eliminate the residual diethylpyrocarbonate.

The sucrose solutions were adjusted to 7 and 47% by adding sterile solutions of NaCl, $MgCl_2$, and Tris-hydrochloride to the final concentrations of the isotonic buffer. Polyuridylic acid sepharose buffer I (PUS buffer I) was 25% formamide, 0.7 M NaCl, 0.01 M EDTA, and 0.05 M Tris-hydrochloride (pH 7.5). PUS buffer II was 90% formamide, 0.01 M EDTA, 0.2% Sarkosyl, and 0.01 M Tris-hydrochloride (pH 7.5). SSC was 0.15 M NaCl, 0.015 M sodium citrate. Sarkosyl buffer consisted of 0.1 M NaCl, 0.01 M Tris-hydrochloride (pH 7.5), 0.001 M EDTA, and 1% Sarkosyl.

Chemicals. Sepharose 4B and Ficoll were obtained from Pharmacia Uppsala, Sweden. Polyvinylpyrrolidone and bovine serum albumin, fraction V, were purchased from Sigma Chemical Co., St. Louis, Mo., and Nonidet P-40 (NP-40) was a gift from Shell Chemical Co. Acrylamide and *N,N'*-methylenebisacrylamide, obtained from Serva, Heidelberg, Germany, were recrystallized as described previously (15). Polyuridylic acid [poly(U)] was obtained from CalBiochem, Los Angeles, Calif., and nitrocellulose filters, type BA 85, were bought from Schleicher and Schüll, Dassel, Germany. Sucrose, RNase free, was purchased from Schwarz-Mann, Orangeburg, N.Y.; diethylpyrocarbonate, Genetron 113 (1,1,3-trichloro-trifluoro-ethane) and sodium dodecylsarcosinate (Sarkosyl) were bought from Serva, Heidelberg. Sodium dodecyl sulfate (Merck, Darmstadt, Germany) was recrystallized from 95% ethanol. Formamide, analytical grade (Merck) was purified as described previously (29). The scintillators 2,5-diphenyloxazole (PPO), and 1,4-bis-(5-phenyloxazolyl) benzene (POPOP) were purchased from Merck, and toluene and methanol were from Baker, Doventer, Holland. All other chemicals and reagents were analytical grade.

Radioisotopes. [5-^{3}H]uridine (specific activity 24 to 28 Ci/mmol) and [^{14}C]sodium formate (50 to 60 mCi/mmol) were obtained from The Radiochemical Center, Amersham, England.

Propagation of cells. Cell stocks were stored frozen in Eagle medium with 10% calf serum and 10% glycerol in liquid N_2. KB cells were grown in Eagle medium supplemented with 10% calf serum, and BHK-21 and HB cells were grown in Dulbecco medium supplemented with 10% tryptose phosphate broth and 10% calf serum. Cells were subcultured routinely twice a week with split ratios of 1 to 10.

Propagation of Ad12. The methods for cultivation of Ad12 in suspension cultures have been described elsewhere (7). Suspension cultures of KB cells were infected with Ad12 at a multiplicity of infection (MOI) of 20 PFU per cell and harvested after 60 h at 37 C. The infected cells were resuspended in 0.02 M Tris-hydrochloride (pH 8.0), and crude extracts were prepared by ultrasonic treatment. The cellular debris was pelleted by centrifugation at 4 C for 15 min at

5,000 × *g*. The extracts were further purified by extraction with Genetron 113, and the virus was purified by three cycles of equilibrium centrifugation in cesium chloride density gradients. The virus was stored frozen as crude extract and purified immediately prior to use.

Assay for Ad12. Plaque assays were carried out on HEK cells in secondary passage as described previously (18). Ad12 (ρ = 1.328 g/cm^3) was identified by its characteristic buoyant density by equilibrium sedimentation in CsCl density gradients (4) in a model E analytical ultracentrifuge (Beckman) or in an SW56 rotor of the Spinco L2-65B preparative ultracentrifuge. Ad2 (ρ = 1.334 g/cm^3) was used as a density marker.

Assay for mycoplasma. At least once weekly, cell cultures were tested for mycoplasma contamination as described by Hayflick (13) with minor modifications. Furthermore, the cells were routinely screened for mycoplasma by electron microscopy. The cultures used for the experiments to be described were found free of mycoplasma by these methods.

Preparation of labeled rRNA as marker. Exponentially growing BHK-21 cells were labeled for 2 days with [^{14}C]sodium formate (10 μCi/ml) in Dulbecco medium supplemented with 10% tryptose phosphate broth and 10% fetal calf serum (DTFC). Radioactive medium was removed, and the monolayer was washed and incubated with fresh DTFC for an additional 6-h period. Subsequently, the cells were harvested and fractionated into nuclei and cytoplasm as described below. The cytoplasmic fraction was extracted with phenol-chloroform-isoamylalcohol as described by Weinberg et al. (32), and the RNA was precipitated with ethanol overnight at −20 C.

Infection of BHK-21 cells with Ad12. BHK-21 cells grown in DTFC were inoculated with CsCl-purified Ad12 at an MOI of 100 to 200 PFU per cell. The medium was removed and the monolayers were washed with PBS. The cells were inoculated with Ad12 diluted in PBS or mock-infected with PBS. After a 2-h adsorption period at 37 C, the inoculum was removed, the cells were washed with PBS, and fresh DTFC was added.

Labeling of infected cells. At different times after infection, as shown in each experiment, infected or mock-infected cells were labeled for 2 h at 37 C with [5-^{3}H]uridine at 400 μCi/ml in DTFC.

Cell fractionation and isolation of polysomes. After the labeling period, the monolayers were cooled to 0 C and washed three times with PBS-d. The cells were scraped off the plate and lysed with isotonic buffer containing 0.5% NP-40 for 10 min at 0 C. Suspensions of nuclear were vortexed five times for 10 s, and nuclei were pelleted by centrifugation for 3 min at 2,000 × *g*. The nuclei pellets were washed once by the same procedure, and both supernatants were pooled to yield the cytoplasmic fraction. To isolate the polysomes, the cytoplasmic fraction was layered on top of a 7 to 47% sucrose density gradient in isotonic buffer and centrifuged at 4 C for 120 to 150 min at 35,000 rpm in an SW41 rotor. In Fig. 1 the result of a typical polysome isolation experiment is presented. Routinely, fractions corresponding to *S*

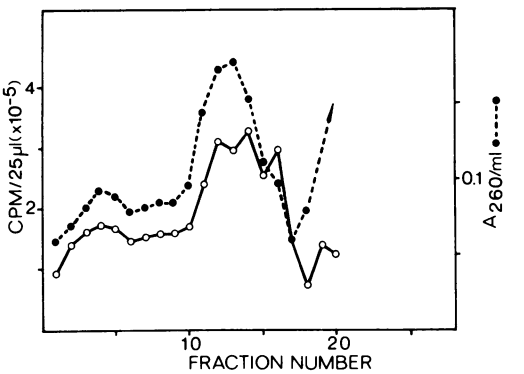

FIG. 1. *Isolation of polysomes from BHK-21 cells infected with Ad12. BHK-21 cells grown on 60-mm plastic dishes were labeled with [5-^{3}H]uridine (400 µCi/ml) between 10 and 12 h postinfection. Subsequently, the cells were fractionated into nuclei and cytoplasm as described in Materials and Methods. The cytoplasmic fraction was centrifuged at 1 C for 120 min at 35,000 rpm on a 7 to 47% sucrose gradient in isotonic buffer. In portions of each fraction the absorbancy at 260 nm (●) and trichloroacetic acid-insoluble radioactivity (O) were determined.*

values higher than 100 were pooled, and the RNA was extracted and subjected to affinity chromatography on poly(U) sepharose.

Poly U sepharose chromatography. Poly(U) sepharose was prepared by the method described by Lindberg and Persson (14) with minor modifications. Dissociation of polysomes and chromatography on poly(U) sepharose was performed as reported by Lindberg et al. (15). RNA molecules (at least in part mRNA) containing sequences of polyadenylic [poly (A)] acid and RNA molecules lacking such sequences could be separated by affinity chromatography on poly(U) sepharose columns. The data presented in Fig. 2 show the elution profile of the [^{3}H]uridine-labeled RNA extracted from polysomes from an experiment similar to the one shown in Fig. 1. Subsequently, both the poly(A)-containing RNA (PUS II fractions) and the RNA without poly(A) sequences (PUS I fractions) were precipitated with ethanol before they were analyzed by velocity sedimentation on sucrose density gradients or by polyacrylamide gel electrophoresis.

Analysis of RNA on sucrose density gradients. The ethanol precipitates of the PUS II fraction were resuspended in 0.1 × SSC and centrifuged on 5 to 20% sucrose density gradients in Sarkosyl buffer at 4 C for 6 h at 35,000 rpm in an SW41 rotor. Portions of each fraction were counted directly, and the RNA from the remaining volume was hybridized to Ad12 DNA fixed on nitrocellulose filters as described below.

Polyacrylamide gel electrophoresis. Polyacrylamide gel electrophoresis in the presence of 98% formamide was carried out essentially as described by Duesberg and Vogt (8). Upper and lower buffers were 0.02 M sodium phosphate (pH 6.8) in water and formamide, respectively. RNA samples in 0.2 ml of a mixture containing 1 volume of buffered formamide, 3

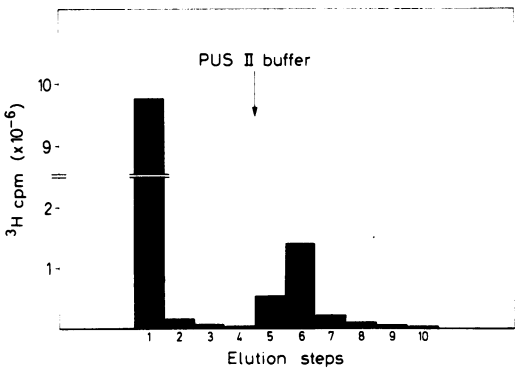

FIG. 2. *Poly(U) sepharose chromatography of polysomal RNA. Polysomes were isolated as described in Fig. 1, dissociated by the addition of Sarkosyl and EDTA to final concentrations of 1% and 0.03 M, respectively, and subsequently diluted five times with PUS I buffer. This RNA solution was passed over a poly(U) sepharose column of 1-ml bed volume at a flow rate of 0.2 to 0.4 ml/min at 4 C. The column was washed three times with 2 ml of PUS I buffer, and the bound RNA was eluted six times with 1 ml of PUS II buffer. The first bar in the graph represents the ^{3}H radioactivity in the percolate of the column.*

volumes of glycerol, and 6 volumes of formamide were applied to 8-cm gels consisting of 3.5% polyacrylamide in buffered formamide. The samples were overlaid with buffered formamide and subjected to electrophoresis for 16 h at 100 V at room temperature. After electrophoresis, the gels were cut into 2-mm slices, and the RNA from each slice was eluted for 24 h at 37 C in 1 ml of hybridization buffer, and hybridized to Ad12 DNA on nitrocellulose filters as described below.

DNA-RNA hybridization. Ad12 DNA was bound to nitrocellulose filters by filtering the DNA solution in 4 × SSC at a rate of ≤5 ml/min. The filters were kept overnight under vacuum and heated for 2 h at 80 C before use.

The DNA-RNA hybridization reactions were carried out with Ad12 DNA bound to nitrocellulose filters (13 or 25 mm in diameter) in 0.3 to 1.6 ml of the RNA solution in 50% formamide, 5 × SSC, and 0.1% sodium dodecyl sulfate, for 3 days at 37 C. After hybridization, the filters were washed with 2 × SSC, treated with RNase A (heated for 10 min at 90 C), 20 μg/ml in 2 × SSC, for 1 h at room temperature, and washed again with 2 × SSC.

For DNA saturation experiments, a series of filters containing 0.5, 1, 2, and 4 μg of Ad12 DNA was used, and the hybridization data were plotted as described by Lucas and Ginsberg (17). The data presented in Fig. 3 show the extent of hybridization of the poly(A)-containing RNA isolated from Ad12-infected BHK-21 cells at various times after infection and from HB cells, an Ad12-transformed BHK-21 cell line, to increasing amounts of Ad12 DNA. From the plot, the saturation hybridization value and the amount of DNA required for saturation were calculated. In all experiments described in this report, DNA-RNA hybridization was carried out under conditions of DNA excess, using 5 μg of Ad12 DNA per filter.

Determination of radioactivity. For direct counting, samples were diluted in a scintillator containing 5.0 g of POP and 0.3 g of POPOP per liter of a 1:1 toluene-methanol mixture. In some experiments, portions of each sample were spotted onto glass fiber filter disks, dried, and counted in a scintillator containing only toluene and POP and POPOP as described.

To determine the trichloroacetic acid-insoluble radioactivity, samples were precipitated in 10% trichloroacetic acid for 10 min at 0 C in the presence of 50 μg of bovine serum albumin per ml as carrier. The samples were passed through glass fiber filters and the filters were washed with cold 5% trichloroacetic acid and ethanol. Subsequently, the filters were dried and counted in the toluene-based scintillator.

Physical determinations. Radioactive samples were counted in a 3385 Tri-Carb liquid scintillation spectrometer. The optical density of samples was measured in a Zeiss PMQ II spectrophotometer. Analytical centrifugations were performed in a Spinco model E analytical ultracentrifuge (Beckman, Inc., Palo Alto, Calif.) equipped with UV optics and a monochromator.

RESULTS

Kinetics of transcription of Ad12 DNA in BHK-21 cells. In earlier work (21, 22), it was demonstrated that transcription of Ad12 DNA in BHK-21 cells starts approximately 10 h

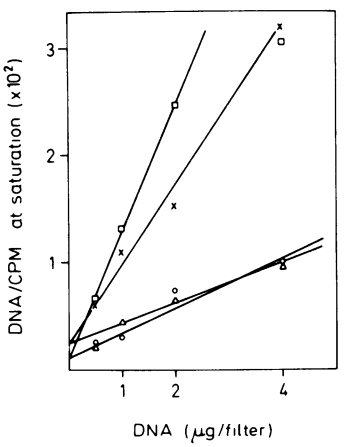

FIG. 3. *Saturation hybridization of viral mRNA with Ad12 DNA. The data are presented as reciprocal plots and are derived from saturation hybridization experiments using poly(A)-containing RNA labeled in Ad12-infected BHK-21 cells at 7 to 9 h postinfection (PI) (circles), 10 to 12 h postinfection (triangles), and 24 to 26 h postinfection (squares). The RNA from HB cells, an Ad12-transformed BHK-21 cell line (crosses), was also analyzed. The RNA inputs per filter were as follows: 7 to 9 h Pi, 134,300 counts/min; 10 to 12 h PI, 129,400 counts/min; 24 to 26 h PI, 115,600 counts/min, and HB cells, 60,350 counts/min. The values on the ordinate are expressed as micrograms of DNA per counts per minute at saturation (reciprocal plot).*

postinfection, and that only the exclusively early genes of Ad12 are transcribed. In this report, the transcription of the Ad12 genome in abortively infected cells was further investigated, and the time course of appearance of viral mRNA on the polysomes was determined.

Virus-specific RNA containing poly(A) sequences was first detected in association with polysomes between 5 and 7 h after infection. Viral RNA was clearly demonstrable between 7 and 9 h after infection, and the amount of viral RNA reached a maximum between 10 and 12 h (Fig. 4). At later times after infection, there was a steady decline in the amount of viral RNA synthesized; however, small quantities of virus-specific RNA continued to be produced late, i.e., 48 to 50 h after infection. As pointed out above, all experiments were performed under saturation conditions for DNA-RNA hybridization.

Characterization of virus-specific mRNA from Ad12-infected BHK-21 cells: analysis by velocity sedimentation on sucrose density gradients. The size distribution of Ad12-specific mRNA synthesized in BHK-21 cells was determined by velocity sedimentation on sucrose density gradients. At various times after infection, the RNA was labeled with [^{3}H]uridine, and the polysome-associated mRNA was isolated and sedimented on sucrose density gradients. The virus-specific RNA was identified by DNA-RNA hybridization.

At the three time points investigated, 7 to 9, 10 to 12, and 24 to 26 h after infection (Fig. 5), the major species of Ad12-specific RNA sedimented at 17 to 18S. This result confirmed the

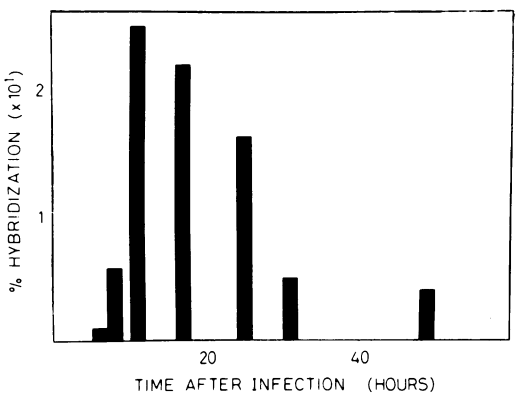

FIG. 4. *Time course of synthesis of Ad12-specific RNA in BHK-21 cells. BHK-21 cells inoculated with Ad12 at a multiplicity of infection of about 200 PFU/cell or mock-infected with PBS were pulse-labeled with [5-^{3}H]uridine at different times after infection as indicated. The cells were fractionated into nuclei and cytoplasm, the RNA was extracted, and mRNA molecules were selected by PUS chromatography as described before. mRNA was analyzed by saturation hybridization with increasing amounts of Ad12 DNA on nitrocellulose filters. The ^{3}H-labeled RNA bound at saturation levels was calculated as described in Materials and Methods and is presented here as percentage of the input radioactivity applied to the filters. Background values of hybridization (usually ≤0.01%) derived from experiments with mock-infected cells were subtracted from all experimental values.*

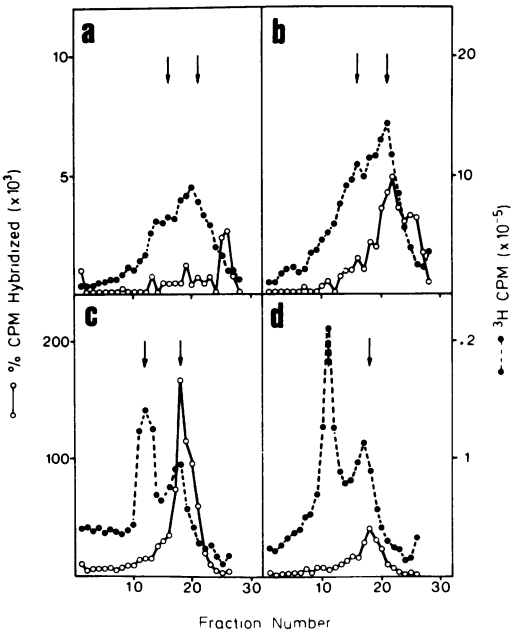

FIG. 5. *Analysis of Ad12-specific RNA by velocity sedimentation on sucrose density gradients. BHK-21 cells grown in 100-mm plastic dishes were inoculated with Ad12 at a multiplicity of infection of about 200 PFU/cell or mock-infected with PBS. Subsequently, the cells were pulse-labeled with [5-^{3}H]uridine at time points as indicated. The poly(A)-containing RNA was purified from the polysomes as described and analyzed by sedimentation on 5 to 20% sucrose density gradients in Sarkosyl buffer. The samples were sedimented for 6 h at 35,000 rpm and 4 C in an SW41 rotor. As marker, [^{14}C]rRNA was added to each gradient; the positions of the 18S and 28S rRNA in the gradients are indicated by arrows. Total radioactivity was determined in 20-μliter portions of each fraction and the remaining RNA was hybridized to Ad12 DNA under conditions of DNA excess (5 μg per filter) on nitrocellulose filters, as described in Materials and Methods. The values of percent counts per minute hybridized, expressed on the ordinate, were calculated according to the formula as follows: (counts per minute hybridized in each fraction × 100)/(total counts per minute recovered from gradient). (a) RNA from uninfected control cells. (b) RNA from Ad12-infected cells labeled 7 to 9 h postinfection. (c) RNA from Ad12-infected cells labeled 10 to 12 h postinfection. (d) RNA from Ad12-infected cells labeled 24 to 26 h postinfection.*

finding of Raška et al. (22). Early and late after infection, minor fractions of mRNA were found sedimenting heterogeneously between 25*S* and 35*S*. Since the RNA used in this study had been denatured by 90% formamide during the elution from poly(U) sepharose, it is unlikely that the fast-sedimenting RNA could be accounted for by aggregation of 17*S* RNA molecules. However, to rule out this possibility and to obtain a more precise estimate of the molecular weight of these RNA species, the viral RNA isolated from abortively infected cells was also analyzed by polyacrylamide gel electrophoresis in 98% formamide.

Analysis by polyacrylamide gel electrophoresis in 98% formamide. The polysome-associated viral mRNA from Ad12-infected BHK-21 cells was also analyzed under conditions that completely denatured RNA. The major size classes of viral mRNA were determined by electrophoresis in polyacrylamide gels in 98% formamide (Fig. 6). The major components had molecular weights of 0.9×10^6 and 0.67×10^6 to 0.65×10^5 at all time periods after infection that were investigated. There was a minor viral mRNA component of 1.5×10^6 daltons whose proportion increased slightly with time after infection. At late times after infection (24 to 26 h), a new size class of 1.9×10^6 daltons could be detected. The small amount of low-molecular-weight RNA of heterogeneous size was probably due to breakdown of viral RNA. It should be emphasized that, due to the sensitivity of this method to detect breaks in the RNA molecules and the high specific radioactivity of the RNA, any delay to perform the

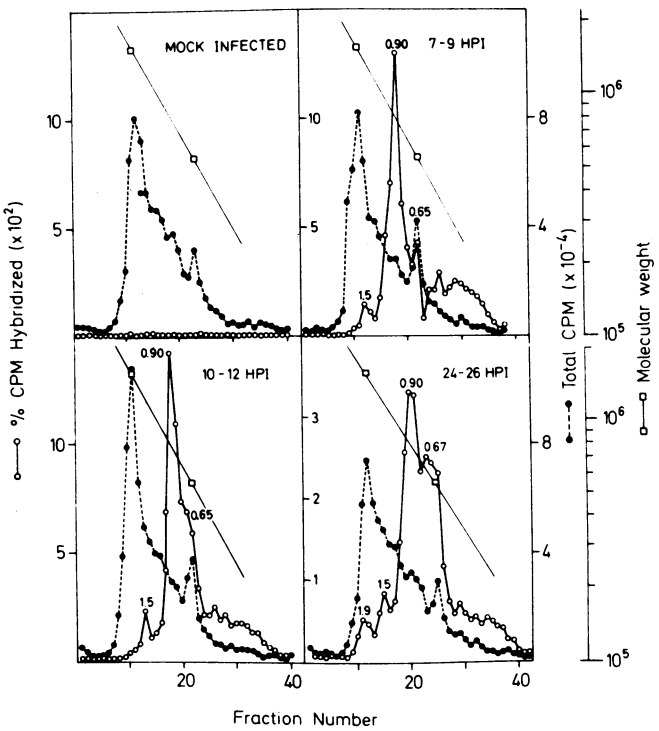

FIG. 6. *Analysis of Ad12-specific RNA from Ad12-infected and mock-infected BHK-21 cells by electrophoresis on polyacrylamide gels in 98% formamide. The polysome-associated, poly(A)-containing RNA was isolated from Ad12-infected BHK-21 cells as described in the legend to Fig. 5. The RNA was analyzed by electrophoresis on polyacrylamide gels in 98% formamide as described in Materials and Methods. As marker, ^{14}C-labeled 18S and 28S rRNA was used. Electrophoresis was performed at room temperature for 16 h at 100 V. Subsequently, the gels were cut into 2-mm slices and the RNA from each slice was eluted with 50% formamide, 5 × SSC, and 0.1% sodium dodecyl sulfate for 24 h at 37 C. The ^3H and ^{14}C activities were counted in one portion of each fraction; another portion was used in DNA-RNA hybridization experiments with Ad12 DNA (5 μg/filter). According to the data presented in Fig. 3, this amount of DNA is well in excess over the viral-specific RNA present in the gel. The values of percent counts per minute hybridized, expressed on the ordinate, were calculated as described in the legend to Fig. 5.*

analysis resulted in an increase in the low-molecular-weight RNA and a concomitant loss of the high-molecular-weight molecules.

Characterization of Ad12-specific mRNA from HB cells. Growing HB cells were pulse-labeled with [5-^{3}H]uridine (400 μCi/ml) for 2 h, and the polysome-associated mRNA was isolated as described in Materials and Methods. In DNA-RNA hybridization experiments, 0.26% of the labeled, poly(A)-containing RNA associated with polysomes was found to be Ad12 specific.

The data presented in Fig. 3 (cf. curve designated by crosses) relate the degree of hybridization of Ad12-specific mRNA from HB cells to the amount of Ad12 DNA on the filter. All experiments described in the following were performed under conditions of DNA excess (5 μg per filter).

The Ad12-specific mRNA from Ad12-transformed BHK-21 cells (HB cells) was also analyzed by polyacrylamide gel electrophoresis in 98% formamide and by DNA-RNA hybridization of each gel fraction (Fig. 7). The main size classes of viral RNA corresponded to molecules of 1.5×10^6 and 0.88×10^6 daltons with minor peak fractions at 0.6×10^6 and 0.47×10^6 daltons.

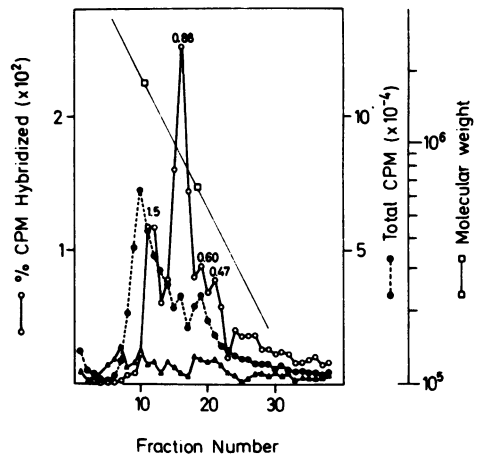

FIG. 7. *Analysis of Ad12-specific RNA isolated from HB cells by electrophoresis on polyacrylamide gels in 98% formamide. RNA pulse labeled for 2 h in HB cells was isolated and analyzed as described in the legend to Fig. 6. The RNA eluted from each gel slice was hybridized to Ad12 DNA on nitrocellulose filters (5 μg of DNA per filter). As shown in Fig. 3, this amount of DNA is in excess over the viral RNA present in the gel. The line represented by triangles shows the result of unspecific hybridization to calf thymus DNA (5 μg per filter). The values of percent counts per minute hybridized, expressed on the ordinate, were calculated as described in the legend to Fig. 5.*

DISCUSSION

Adenovirus type 12 infects BHK-21 cells abortively. At 20 min after inoculation, viral particles can be readily detected in the cytoplasm of the infected cells by electron microscopy (D. T. Brown and W. Doerfler, unpublished results). At 2 h postinfection, an Ad12-specific complex of DNA, RNA, and protein can be isolated (7). The data presented in this communication showed that virus-specific RNA associated with polysomes could first be detected between 5 and 7 h postinfection. The amount of newly synthesized viral mRNA was maximal at 10 to 12 h postinfection and then decreased, but was still detectable as late as 48 to 50 h postinfection. The reasons for this decrease in the rate of viral mRNA synthesis are not understood. One possible explanation is that the fragmentation of cellular DNA which took place late after infection (4) altered cellular functions which may be responsible for viral transcription. The synthesis of T-antigen starts as early as 8 h postinfection (24). Therefore, if the T-antigen is indeed a virus-coded function, its time of synthesis correlates fairly well with the onset of viral mRNA synthesis.

Viral mRNA has been characterized by polyacrylamide gel electrophoresis in the presence of 98% formamide. This technique represents the most reliable method for the determination of molecular weights of RNA molecules, since both intermolecular aggregation and intramolecular interactions are negligible under these conditions (8). Viral mRNA has been analyzed both in abortively infected BHK-21 cells at different times after infection and in BHK-21 cells transformed by Ad12, the HB line. This line has been isolated by W. A. Strohl et al. (28). HB cells contain many genome equivalents of Ad12 DNA as shown by the technique of reassociation kinetics (E. Fanning and W. Doerfler, unpublished results).

The results of gel electrophoresis experiments in the presence of 98% formamide demonstrate that the viral mRNA in BHK-21 cells abortively infected with or transformed by Ad12 is very similar in size. The main size classes of viral mRNA had molecular weights of 0.88×10^6 to 0.90×10^6 and 0.65×10^6 to 0.67×10^6. A minor fraction of 1.5×10^6 daltons consistently occurred in abortively infected cells and increased with time after infection. At 24 to 26 h postinfection, there was also a minor peak at 1.9×10^6 daltons. In Ad12-transformed cells, the RNA fraction of 1.5×10^6 daltons was very pronounced.

It has not yet been shown that the different size classes of viral mRNA are indeed distinct species with nonoverlapping sequences. If this assumption were correct, about 30% of the transcriptional capacity of the Ad12 genome would be expressed in cells abortively infected with, or transformed by, adenovirus type 12. The pattern of Ad12-specific mRNA isolated from KB cells at early times after productive infection with Ad12 is almost identical to the distribution of Ad12-specific mRNA isolated from HB cells (K. H. Scheidtmann, J. Ortin, and W. Doerfler, unpublished results).

At this time, it is uncertain whether the Ad12-specific transcripts in abortively infected and transformed hamster cells are derived from integrated viral genomes (3, 5) or from free viral DNA.

To distinguish between these two possibilities, a study has been initiated to determine the specific regions of the viral genome from which these RNA molecules have been transcribed. Mulder et al. (19) have demonstrated that Ad12 DNA is cleaved to six unique fragments by the restriction endonuclease Eco $R \cdot R_I$. These fragments can be separated by electrophoresis on polyacrylamide agarose gels. Preliminary results (J. Ortin and W. Doerfler, unpublished results) indicate that the polysome-associated RNA from Ad12-infected BHK-21 cells anneals preferentially with fragments A (left molecular end) and C (right molecular end) of Ad12 DNA.

In this context it is interesting to note that Sambrook et al. (23) have reported that, in several lines of adenovirus type 2- and adenovirus type 5-transformed rat cells, fragments of viral DNA corresponding to the right and left molecular ends of the viral genome persist. Thus, it will be interesting to compare the patterns of transcription of the viral genome in abortively infected and in transformed cells.

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