Adenovirus Transcription

IV. Synthesis of Viral-Specific RNA in Human Cells Infected with Temperature-Sensitive Mutants of Adenovirus 5

S. M. BERGET,* S. J. FLINT, J. F. WILLIAMS, AND P. A. SHARP

Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, and MRC Institute of Virology, Glasgow G11 5JR, Scotland

Received for publication 26 February 1976

Cytoplasmic RNA sequences produced in HeLa cells infected with the adenovirus 5 temperature-sensitive mutants ts1, ts2, ts9, ts17, ts18, ts19, ts20, ts22, ts49, ts36, and ts125 were characterized by hybridization to DNA probes generated by strand separation of restriction endonuclease fragments of adenovirus 5 DNA. Two "early" mutants defective in DNA synthesis, ts125 and ts36, fail to make wild-type levels of all previously reported classes of late RNA at the nonpermissive temperature. At 40.5°C, both ts125 and ts36 synthesize a wild-type complement of early cytoplasmic RNA 16 h after infection. Under these conditions, no "late" cytoplasmic RNA sequences were observed. Similarly, nuclear RNA present in these cells resembled early cytoplasmic RNA rather than late nuclear RNA. All the late adenovirus 5 temperature-sensitive mutants synthesized normal wild-type levels of late cytoplasmic RNA at the nonpermissive temperature, except ts2, which appears to overproduce certain cytoplasmic species.

During infection of human cells by either adenovirus 5 (Ad5) or adenovirus 2 (Ad2) an ordered sequence of events can be observed which culminates in the synthesis and release of progeny virus particles. In the early stage of infection, defined as occurring before the onset of viral DNA replication, both virus-specific RNA and antigens can be detected in the cytoplasm (9, 10). "Early" RNA appears to be a subset of the total viral RNA produced in a normal infection. (See reference 28 for a recent review of adenovirus transcription.) The use as probes of specific subgenomic fragments of viral DNA produced by digestion with various restriction endonucleases has defined this early RNA as representing 23% of the coding capacity of the genome and mapping at four distinct regions on the genome (3, 20, 29; Flint, Berget, and Sharp, Virology, in press). Several early viral polypeptides have also been described and the regions of the genome coding for some of them identified (11, 21, 25; J. B. Lewis, personal communication). One of these, an Ad5 DNA-binding protein of molecular weight 72,000, has also been defined by mutation (34).

The onset of viral DNA synthesis defines the beginning of the late stage of infection during which almost all of the viral genome is transcribed into stable cytoplasmic RNA, and viral structural proteins accumulate in the nucleus (2, 9, 23, 29, 31, 36). At 18 h after infection most polysomal RNA is transcribed from a viral template (32). This is in contrast to the situation at early times, when only 4% of the RNA bound to polysomes is viral specific (10). Nearly 20 viral peptides (many of which have been identified as the product of a particular region of the genome (5, 14) can be detected in late infected cells. In addition, mutants of several of these late proteins have been isolated and partially characterized (8, 37, 42).

Nuclear RNA has been reported to contain sequences not transported to the cytoplasm during both the early and "late" periods of infection (15, 18, 19, 29, 35). Thus, the processing of nuclear RNA has been suggested as a possible mechanism of control operating to regulate viral transcription in adenovirus-infected cells. In addition, regulation may also be present at the transcriptional level such that different genes are copied at different frequencies, as in Simian Virus 40-infected cells (1). Regardless of what mechanism selects those early sequences eventually transported to the cytoplasm, some alteration in control must occur during the period of DNA replication to achieve the switchover to the production of late sequences. Presumably, viral functions are involved in this process. Those early functions involved in DNA replication have already been implicated as being required for the appearance of late transcription by extrapolation from experi-
ments with DNA synthesis inhibitors such as cytosine arabinoside and fluorodeoxyuridine (17), in which addition of the drug to infected cells prevents both replication and late transcription. In addition to replication proteins, other viral functions may be involved in transcriptional control.

Recently, various temperature-sensitive (ts) mutants of Ad5 have been isolated (4, 40). These mutants have been characterized with respect to complementation group, antigenic phenotype, protein synthesis, and DNA synthesis (Table 1) (22, 24, 38, 41). Using mapping techniques described previously (29), we have examined 11 such mutants for alterations in late RNA synthesis under both permissive and nonpermissive conditions. The method used utilizes single-stranded 32P-labeled DNA probes produced by strand separation of fragments generated by cleavage of viral DNA by restriction endonucleases and yields, not only information about what classes of RNA are present in a given sample, but also an estimate of the amount of each class present.

MATERIALS AND METHODS

Cells. Human HeLa cell lines were grown as described previously (7).

Viruses. Wild-type (wt) Ad5, originally obtained from J. Williams, was grown in suspension HeLa cells and assayed by plaque formation on monolayers of HeLa cells (39).

The Ad5 ts mutants ts1, ts2, ts9, ts17, ts18, ts19, ts20, ts22, ts36, and ts49 (40) were grown on monolayers of HeLa cells at 32.5°C. All stocks were plaque purified on HeLa monolayers before subsequent passage; titers were determined on monolayers of HeLa cells at 32.5°C and at 38.5°C after each passage. With the exception of ts36, all stocks titrated at least 10-fold higher at the permissive temperature, 32.5°C. The titer of ts36 at 38.5°C was 1% of that at 32.5°C. The Ad5 mutant ts125 was provided by H. S. Ginsberg, Columbia University, New York, and was grown and titered as the above mutants.

Preparation of 32P-labeled Ad5 DNA fragments. 32P-labeled Ad5 or Ad2 DNA (0.5 × 106 to 1.5 × 106 counts per min/μg) was prepared from HeLa suspension cells 48 h after infection as described by Sharp et al. (29). Restriction endonuclease EcoR1 was purified from Escherichia coli RY13 according to R. N. Yoshimori (Ph.D. thesis, Univ. of California, San Francisco, 1971). BamH1 from Bacillus amyloliquefaciens was a generous gift of R. Roberts. Viral DNA was digested with restriction endonucleases, and the resulting fragments were separated and eluted from 1.4% agarose gels as indicated previously (30). Each fragment preparation was denatured and layered onto a 0.7% agarose gel. Electrophoresis was carried out for 8 h. Individual 32P-labeled strands were recovered from the agarose gels, degraded to an appropriate size for hybridization experiments (29), and self-annealed to allow renaturation of all contaminating complementary DNA sequences. Single-stranded DNA was selected by passage over hydroxyapatite as previously described (29). The restriction endonuclease fragments used in this study are mapped on their respective genomes in Fig. 1.

Extraction of RNA. Monolayers of HeLa cells were infected with either wt Ad5 or any of the various ts mutants as follows: medium was removed from the plates, virus in phosphate-buffered saline

<table>
<thead>
<tr>
<th>Class</th>
<th>Mutant</th>
<th>Complementation group</th>
<th>Map position</th>
<th>Antigenic phenotype</th>
<th>Possible protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>18</td>
<td>H</td>
<td>44–59 or 69–80†</td>
<td>All capsid antigens made</td>
<td>?</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>I</td>
<td>0–43</td>
<td>All capsid antigens made</td>
<td>?</td>
</tr>
<tr>
<td></td>
<td>49</td>
<td>M</td>
<td>30–30†</td>
<td>All capsid antigens made</td>
<td>?</td>
</tr>
<tr>
<td>II</td>
<td>36</td>
<td>N</td>
<td>58.5–70†</td>
<td>No DNA; no capsid antigens</td>
<td>44K or 15K†</td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>–</td>
<td></td>
<td>No DNA; no capsid antigens</td>
<td>72K DNA-binding protein†</td>
</tr>
<tr>
<td>III</td>
<td>9</td>
<td>G</td>
<td>86–100†</td>
<td>Fiber antigen negative</td>
<td>Fiber†</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>K</td>
<td>86–99†</td>
<td>Fiber antigen negative</td>
<td>Fiber†</td>
</tr>
<tr>
<td>IV</td>
<td>17</td>
<td>D</td>
<td>69–80†</td>
<td>Hexon antigen negative</td>
<td>100K†</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>E</td>
<td>69–80†</td>
<td>Hexon antigen negative</td>
<td>100K†</td>
</tr>
<tr>
<td>V</td>
<td>1</td>
<td>A</td>
<td>69–80†</td>
<td>Hexon transport negative</td>
<td>100K†</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>F</td>
<td>44–59†</td>
<td>Hexon transport negative</td>
<td>Hexon†</td>
</tr>
</tbody>
</table>

* Williams and Ustacelebi (41).
† Russell et al. (22).
‡ Wilkie et al. (38).
§ Williams et al. (42).
‖ Sambrook et al. (27).
J. B. Lewis, personal communication.
‡ Sambrook, personal communication.
* Ginberg et al. (8).
' Lewis et al. (14).
was added, the virus was adsorbed to the cells for 1 h at 37°C, virus was removed, the plates were washed once with prewarmed medium, and after the final addition of medium the plates were incubated at either 32.5 or 38.5°C for an appropriate time. HeLa cells in suspension were infected with an adsorption period of 30 min at 37°C before dilution with prewarmed medium and incubation at 32.5, 38.5, or 40.5°C. The time of addition of medium after adsorption is taken as zero time of infection. For preparation of early RNA, cytosine arabinoside (20 μg/ml) was added to the culture medium, and the cells were harvested 8 h later at 38.5°C and 16 h later at 32.5°C. Late RNA was harvested at 16 h at 40.5°C, at 17 h at 38.5°C, and at 34 h at 32.5°C. Harvest times were selected on the basis of growth curves at various temperatures of ts1, ts36, and wt Ad5; this set of times yielded similar amounts of RNA at 32.5°C and 38.5°C for a wt infection. After lysis of the cells with Nonidet P-40, nuclear and cytoplasmic fractions were isolated (12), from which RNA was prepared as described previously (29). All RNA samples were digested with DNase I (Worthington Biochemical Corp.) and chromatographed over G-75 Sephadex to remove oligonucleotides (26).

Hybridizations. Hybridization conditions used were those that have been described previously (29). All hybridizations were assayed by hydroxylapatite chromatography (26).

RESULTS

Precise mapping on the physical genome of the viral sequences transcribed into RNA in lytically adenovirus-infected cells has become possible with the use of probes of separated strands of individual restriction endonuclease cleavage fragments of 32P-labeled viral DNA (6, 20, 31). RNA sequences present both early and late after infection with either Ad2 (6, 20, 29, 31) or Ad5 (Flint et al., Virology, in press) have been examined in this manner. In this paper we report the results of a survey of RNA synthesis after infection with a collection of Ad5 ts mutants. The procedure used involves the hybridization of 32P-labeled, single-stranded subgenomic viral DNA fragments to unlabeled RNA isolated from the nuclei or cytoplasm of mutant-infected cells. At high RNA concentrations, the percentage of label entering hybrid molecules indicates the fraction of the fragment strand transcribed into cytoplasmic RNA. The amount of probe used in each hybridization mixture was between 10^-4 to 10^-3 μg/ml; therefore, at subsaturating concentrations of total RNA, the hybridizations are RNA driven. Hence, the relative concentration of total RNA required to achieve a particular percentage of hybridization is roughly proportional to the relative amount of complementary RNA in each sample. Thus, the method used not only yields information about the classes of RNA produced in a permissive compared to a nonpermissive infection but also affords an estimation of the quantity of sequences of a particular class present under each condition.

Fig. 1. Cleavage of Ad2 or Ad5 DNA by restriction endonucleases EcoR1, Hpa I, or BamHI. The solid horizontal line represents the genome of either virus. Vertical lines and numerical coordinates above the line indicate sites of cleavage. Letters below the line show the nomenclature used for each fragment generated by cleavage. These data are taken from Mulder et al. (16) and Roberts et al. (manuscript in preparation).
To ascertain whether small amounts of RNA could be quantitatively recovered from mutant-infected cells which have reduced viral RNA synthesis, wt Ad5-infected cells harvested at 16 h after infection at 37°C were counted and mixed 1:1 and 1:4 with uninfected cells. Cytoplasmic RNA was prepared from these mixtures and hybridized to a single-stranded DNA probe prepared by the digestion of 32P-labeled Ad5 DNA with EcoRI. Figure 2 indicates that at subsaturating RNA concentrations, the observed level of hybridization corresponds to the original dilution of the infected cells.

Early mutants. Table 1 shows several known properties of the mutants used in this study. On the basis of these characteristics, two of the eleven mutants synthesize no progeny DNA at the nonpermissive temperature and thus can be considered to have defects in early gene products. These are the class II mutants, ts36 and ts125. Ts125 has been characterized as being defective in the production of an active single-stranded DNA-binding protein, similar to the gene 32 protein of bacteriophage T4 (34); ts36 cannot transform rat cells at the nonpermissive temperature (42), but the viral polypeptide defective in this mutant has not been identified. Late cytoplasmic RNA was prepared from ts36-, ts125-, and wt Ad5-infected HeLa cells maintained at a nonpermissive temperature of 40.5°C for 16 h and hybridized to separated l and r strands of the EcoRI fragments of Ad5 DNA. The saturation values given for either early or late wt Ad5 RNA are interpreted according to the map in Fig. 3 (Flint et al., Virology, in press). As seen in Fig. 4, the two early mutants display essentially identical patterns (Fig. 4A, B) which are very different from a wt pattern (Fig. 4C). For each of the mutants, the fraction of probe entering hybrid at saturating RNA concentrations is not only unlike that obtained with late wt RNA, but is basically similar to the values obtained with RNA prepared at early times from wt Ad5-, ts36-, or ts125-infected cells maintained at the permissive temperature (32.5°C) in the presence of cytosine arabinoside (Fig. 4D, E, F). Thus, both mutants which cannot synthesize viral DNA under nonpermissive conditions appear to make only early cytoplasmic RNA under the same conditions. In addition, both mutants appear to make a normal early complement of RNA when maintained at 38.5°C for 8 h in the presence of cytosine arabinoside (data not shown).

The actual definition of early and late adenovirus RNA has been derived through the use of chemical inhibitors of DNA synthesis such as

![Fig. 2. Hybridization of cytoplasmic RNA prepared from mixtures of uninfected HeLa cells and late Ad5 wt-infected HeLa cells to an EcoRI-produced fragment probe. The fraction of the r strand of 32P-labeled Ad5 EcoRI fragment B entering RNA:DNA hybrid is shown after annealing to cytoplasmic RNA prepared from infected cells mixed 1:4 (▲), 1:1 (●), or not at all (○) with uninfected cells before lysis with Nonidet P-40.](http://jvi.asm.org/)

![Fig. 3. Map of early and late Ad5 RNA sequences. The light arrows represent early sequences and the heavy arrows represent exclusively late sequences and are drawn in 5' to 3' polarity of the RNA transcript (Flint et al., Virology, in press). The r and l strands are transcribed to the right and left, respectively, on the conventional map.](http://jvi.asm.org/)
cytosine arabinoside; the finding that the DNA-negative mutants also appear to produce the same classes of RNA as the drug-treated infected cells reinforces the existence of a distinct early class of viral RNA present before the onset of viral DNA replication. In fact, normal wt early plateau values under saturating RNA conditions can be observed in Fig. 4A and B, suggesting little or no leakage of late sequences to the cytoplasm at 40.5°C. A rough calculation of an upper limit for the concentration of late RNA which could be present but not detectable in Fig. 4A and B can be obtained by the following comparison. For an Ad5 EcoRI B r strand probe, the concentration of late cytoplasmic RNA at half-saturation (28% of the labeled probe as hybrid) is less than 5 µg/ml (Fig. 4C). Late RNA prepared from either ts125- or ts36-infected cells at 40.5°C drives 20% of the same labeled probe into hybrid at saturating RNA concentrations (0.40 to 2.0 or 0.40 to 1.0 mg/ml, respectively; Fig. 4A and B). If late RNA sequences represented 0.25% of the RNA present at the highest RNA concentration used in these saturations, 5 µg of late RNA per ml would have been present in these samples. As indicated above, this would drive 28% of the probe into hybrid, significantly more hybridization than was observed. Therefore, less than 0.25% of wt levels of late sequences complementary to the r strand of Ad5 EcoRI B are present in ts36- and ts125-infected cells at the nonpermissive temperature. Similar analyses have been done for the other probes used yielding estimated late RNA leakages equivalent to that calculated above.

When RNA was prepared from ts36- and ts125-infected cells maintained at 32.5 or 38.5°C for 34 or 17 h, respectively, the results plotted logarithmically in Fig. 5 were obtained. At 32.5°C, both mutants do produce normal wt levels of late cytoplasmic RNA as seen by comparing the hybridization value obtained at saturating concentrations of RNA shown in Fig. 5A and B with those shown in Fig. 4C for wt Ad5. At 38.5°C, in contrast to the results ob-
tained at 40.5°C, some production of late cytoplasmic RNA is apparent.

The above data described the production of cytoplasmic RNA in ts36- or ts125-infected cells and thus does not address the question of whether or not late RNA is produced in the nuclei of such cells at nonpermissive temperatures, but is never exported to the cytoplasm. Figures 4G, H, and I show the hybridization of the six Ad5 EcoRI probes to nuclear RNA prepared from the same ts36-, ts125-, and Ad5 wt-infected cells for which the cytoplasmic RNA pattern is shown in Fig. 4A, B, and C, respectively. The pattern exhibited by both mutants is strikingly different from that of wt Ad5. Whereas 100% of the r strand of viral DNA appears to be transcribed into late nuclear wt RNA, only 25% of the r strand is represented in either the ts36 or ts125 late nuclear RNA. The 1 strand material is similarly reduced in the mutants with respect to wt. Indeed, the observed mutant patterns resemble early cytoplasmic RNA patterns and not late nuclear patterns. Although the saturation values obtained for mutant nuclear RNA are quite similar to those observed for cytoplasmic RNA with the r strand of fragments EcoRI B and C, the nuclear saturation values for the other three early regions

FIG. 5. Hybridization of 32P-labeled separated strands of the three EcoRI fragments of Ad5 DNA to RNA from ts36 and ts125-infected cells. Cytoplasmic RNA from ts36 (A)- or ts125 (B)-infected HeLa monolayers prepared at 34 h at 32.5°C or at 17 h at 38.5°C was annealed to r strand (32.5°C [●], 38.5°C [○]) and 1 strand (32.5°C [▲], 38.5°C [△]) probes.
of the genome (see Fig. 3) are approximately 10% greater than the equivalent cytoplasmic levels.

Late mutants. Nine of the mutants listed in Table 1 have an altered gene product required late in the infective cycle. Among the nine are members of each of the four late antigenic classes as originally defined by Williams et al. (42). The nine mutants complement each other, although it is doubtful if this indicates that each mutant defines a separate viral protein.

The class I mutants ts18, ts19, and ts49 appear to define different viral polypeptides, although in no case has the identity of the defective protein been reported. The three mutants complement each other well and map in distant areas of the genome (42). By definition, class I mutants appear to synthesize active late antigens at the nonpermissive temperature and thus the three mutants might be predicted to synthesize normal quantities of late RNA. Indeed, as indicated in Fig. 6C and F, both ts18 and ts49, respectively, appear to make essentially normal amounts of all classes of late RNA.

Two group III mutants, ts9 and ts22, were included in this study. Both are probably located in the structural gene for the viral fiber polypeptide(s) (42). As might thus be predicted, both also appear to synthesize wt levels of late RNA at the nonpermissive temperature (ts9, Fig. 6A; ts22 not shown).

There are several mutants defective in the normal production of hexon, the major viral structural protein. These include ts1, ts2, ts17, and ts20, which have been assigned to either the antigenic class IV or V on the basis of the subcellular location of hexon antigen at the nonpermissive temperature (22). The four mutants do complement (21) on the basis of a 10-fold increase in titer upon mixed infection under nonpermissive conditions, although it is not clear that four individual polypeptides are defined by the four mutations. Genetic mapping indicates that ts2 maps furthest leftward of the four, and ts1 maps furthest to the right (42). Physical mapping studies suggest that the area around ts2 may include the hexon structural gene (27). ts1, ts17, and ts20 map quite close to

![Image](http://jvi.asm.org/)

**Fig. 6.** Hybridization of 32P-labeled separated strands of the six EcoRI fragments of Ad5 DNA to RNA from Ad5 late mutant-infected cells. Cytoplasmic RNA from ts9 (A)-, ts17 (B)-, ts18 (C)-, ts19 (D)-, ts20 (E)-, ts49 (F)-, and wt Ad5 (G)-infected HeLa monolayers prepared at 34 h at 32.5°C or at 17 h at 38.5°C was annealed to r strand (32.5 C [●], 38.5 C [○]) and I strand (32.5 C [▲], 38.5 C [△]) probes.
one another and in an area of the genome bounded to the left by the gene for the DNA-binding protein which is deficient in ts125 and to the right by the fiber protein gene (27, 42). Late mRNA selected by hybridization to a fragment from this region stimulates the synthesis of a 100,000-dalton polypeptide when added to an in vitro protein translation system (14). Hence, one or more of these mutants might carry a lesion in the 100,000-dalton protein. A viral polypeptide of that molecular weight has also been reported to be located on polysomes late after infection (12). The function of this polypeptide has not been reported, but its tenacious affinity for polyadenylic acid-containing mRNA suggested a possible involvement in late viral RNA production. However, when ts1, ts17, and ts20 were examined for late RNA production (ts1 not shown; ts17, Fig. 6B; and ts20, Fig. 6E), all three produced essentially identical wild-type patterns. Thus, it appears that if ts17, ts20, or ts1 are mutants in the gene for the 100,000-dalton polypeptide, this polypeptide is probably not involved in late viral RNA metabolism.

When late RNA was prepared from ts2-infected cells at 32.5 or 38.5°C, a surprising result was obtained. Not only did ts2 produce late RNA at the nonpermissive temperature, but it appeared to do so at a greater level than that observed at the permissive temperature. Late RNA extracted from ts2-infected cells maintained at 32.5°C or 38.5°C was hybridized to a variety of adenovirus DNA probes covering the length of the genome (Fig. 7). To cover left-end sequences in more detail than is possible using the Ad5 EcoRI A fragment as a probe, hybridizations were performed using the BamHI fragments B, C, and D and the EcoRI fragment B of the closely related serotype Ad2 (Fig. 2). Beginning at the left end of the genome, RNA annealing to the r strand of BamHI B is present at roughly the same concentration at both permissive and nonpermissive temperatures and the sequences hybridizing to the l strand of this fragment may be even slightly reduced at

---

Fig. 7. Hybridization of cytoplasmic RNA from HeLa monolayers infected with ts2 prepared at 34 h at 32.5°C and 17 h at 38.5°C to a variety of adenovirus DNA probes. The fraction 32P-labeled separated strand entering RNA:DNA hybrid is shown for r strand (32.5°C [•], 38.5°C [○]) and l strand (32.5°C [▲], 38.5°C [△]) probes prepared by digestion of Ad2 DNA with BamHI (BamHI fragments B, C, and D are shown in panels A, C, and B, respectively) and EcoRI (EcoRI fragment B in panel D) or by digestion of Ad5 DNA with EcoRI (EcoRI fragments B and C in panels E and F, respectively).
38.5°C (Fig. 7A). From BamHI D rightward to the end of the genome, sequences complementary to r-strand DNA appear to be in higher abundance at the nonpermissive temperature (Fig. 7B-F). 1-strand sequences, where present, appear in similar concentrations at both temperatures for these same fragments. The greatest difference in r-strand-sequence abundancies at the two temperatures appear to occur in Ad2 BamHI C and Ad2 EcoRl B, mapping from 40.9 to 59.0 and 58.5 to 70.7, respectively, on the genome. Thus, the over-production extends to only certain areas of the genome. Experiments using nuclear RNA also indicate similar higher abundancies of certain late regions at 38.5°C (data not shown).

DISCUSSION

Separated strands of restriction endonuclease cleavage fragments of viral DNA have been used to map complementary RNA from both transformed and lytically infected cells (29; Flint et al., Virology, in press). In this study similar probes were used to follow the synthesis of RNA after infection with ts mutants of Ad5. Both the region of the genome transcribed and the relative abundancy of the transcript were determined. When separated strands of smaller fragments are used as probes, the analysis is probably monitoring the production of single RNA's. If strands of larger fragments are used, only the synthesis of groups of mRNA's can be followed. The EcoRl B and C fragments are small, representing 16% and 7.3% of the genome, respectively, whereas the EcoRl A fragment is particularly large, covering 76.7% of the genome. Hence, the EcoRl B and C fragments may be template for only a few mRNA's. The evidence that cells infected with a mutant yield RNA which saturates these probes at wt plateau values suggest that a mRNA from this area of the genome is not missing under our nonpermissive conditions. Because of the size of the Eco R1 A fragment, 26,500 base pairs, however, the fraction of probe entering hybrid with saturating concentrations of RNA would be insensitive to the absence of one or two mRNA's of 1,000 bases in length. Hence, the statement that various mutants produce wt levels of RNA must be understood in light of this inherent limitation. We have also shown in Fig. 2 that these RNA-driven hybridization re- actions are useful for following twofold changes in the relative abundancy of complementary RNA. Such small differences, however, do strain the technique, and only differences of a factor of 5 or greater should be considered as meaningful.

Of the 11 mutants examined, only the two early mutants, ts36 and ts125, did not induce the synthesis of late viral cytoplasmic RNA at nonpermissive temperatures. Cells infected with these mutants and maintained at 40.5°C for 16 h produced normal levels of all early mRNA's but failed to synthesize detectable late mRNA's at a level more than 0.25% of the normal level for this group of mRNA's. Thus, whether the onset of viral DNA synthesis is blocked by mutation or by the addition of chemical agents such as cytosine arabinoside, the same select early mRNA's are exported to the cytoplasm. When early total cell RNA or nuclear RNA is prepared from Ad2 wt-infected cells that have been treated with 20 μg of cyto- sine arabinoside to inhibit DNA synthesis, viral RNA sequences can be detected in the nucleus that are not exported to the cytoplasm. It is interesting that we failed to detect many such exclusively nuclear viral RNA sequences in ts36- or ts125-infected cells (Fig. 4G, and H). In these experiments low multiplicities of infec- tion were used (1 to 2 PFU/cell) to minimize mutant leakage. Previous experiments with wt virus used rather high multiplicities of infec- tion to maximize detection of complementary RNA, and perhaps the difference between the viral RNA content of the mutant and wt early nuclear RNA is a result of the relative multi- plicity of infection. In any case, cells infected with either early mutant do not grossly accumulate viral RNA sequences other than cyto- plasmic ones in their nuclei.

Within the limits discussed above, none of the late mutants examined are deficient in either the gross production of late mRNA or in the production of specific late messages. Interest- ingly, at the nonpermissive temperature, ts2-infected cells seem to overproduce viral RNA from the region of the r strand extending from the middle of BamHI D to the right end of the genome with the predominant region of overproduction mapping in Ad2 BamHI C and Ad2 EcoRl B. ts2-infected cells are known to be deficient in the production of hexon protein (42), and the ts lesion in this mutant maps in the region of the genome coding for the hexon protein, from 44 to 59 on the genome within Ad2 BamHI C. Whether the underproduction of hexon protein is related to the apparent overproduction of this group of late mRNA sequences is not known. The simplest explanation for such concurrent overpro- duction of a large group of mRNA's is regulation at one promotor site for transcription for this complete region of the genome. We do not at the moment know if a shift of ts2-infected cells from
low temperature to high temperature will immediately give a higher rate of transcription than that observed with cells infected with wt virus; these experiments are in progress.

Addendum

Using different techniques T. H. Carter and H. S. Ginsberg (J. Virol. 18:156-166) have recently reported results consistent with the data presented here for ta125 and ta149 (isogenic to ta68).

ACKNOWLEDGMENTS

We thank Janice Haverty for her technical help.

S. M. Berget was supported by a fellowship from the National Cancer Institute (I P22 NCI02391-01), and S. J. Flint was supported by a fellowship from the Science Research Council of Great Britain. P. A. Sharp gratefully acknowledges an American Cancer Society faculty grant. This work was funded by Public Health Service grant NCI 13106-03 from the National Cancer Institute and grant no. VC-151 from the American Cancer Society.

LITERATURE CITED


32. Thomas, D. C., and M. Green. 1966. Biochemical studies on adenovirus multiplication. XI. Evidence of a cytoplasmic site for the synthesis of viral-coded pro-


