JOURNAL OF VIROLOGY, Oct. 1982, p. 426–430
0022-538X/82/100426-05$02.00/0
Copyright © 1982, American Society for Microbiology
Vol. 44, No. 1

Normal Translation of Human Adenovirus mRNA in Cell-Free Lysates Prepared from Abortively as Well as Productively Infected Monkey Cells

MARGARET P. QUINLAN[1]† AND DANIEL F. KLESSIG[1,2]*

*Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724,[1] and Department of Cellular, Viral and Molecular Biology, University of Utah, Salt Lake City, Utah 84112[2]**

Received 9 April 1982/Accepted 24 June 1982

A variety of mRNAs, including adenovirus-specified mRNAs isolated from infected human cells, were translated with similar efficiencies in S10 cell-free lysates prepared from productively and abortively infected monkey cells. These results may suggest that reduced synthesis of the late viral proteins in abortively infected monkey cells is not due to a defect in the protein-synthesizing apparatus of the cell.

The block to the multiplication of human adenovirus in monkey cells presents a convenient system in which to study the mechanisms used by eucaryotic cells to control the expression of incoming viral genes as well as perhaps that of their own genes. Because this block can be overcome by an alteration in the DNA-binding protein of the infecting adenovirus (12) or by coinfection with simian virus 40 (SV40) (19), the sequence of the events which lead to a productive or an abortive infection can be studied within the same cell line.

Although the expression of the viral early genes (1, 6, 23) as well as the replication of viral DNA (9, 20) occur normally in abortively infected monkey cells, the synthesis of several late proteins, particularly of fiber protein, is dramatically reduced (7, 8, 10). This diminished synthesis is at least partly due to a reduction in the level of viral late mRNA in the abortively infected monkey cells (5, 10). That this may not, however, be the entire explanation is suggested by analyzing the levels of the fiber protein and its mRNA. Although the synthesis of the protein in vivo is reduced by 100- to 1,000-fold, the RNA as measured by C_rt or Northern blot analysis is diminished only 5- to 20-fold in abortive infections (10, 11).

To determine whether the poor utilization of the residual fiber RNA made in abortively infected cells is due to a defect in the translational apparatus of these cells, we compared cell-free translation lysates prepared from monkey (CV_1) and human (HeLa) cells. S10 lysates prepared essentially as described by Celma and Ehrenfeld (2) from uninfected CV_1 monolayers or HeLa suspension cultures were treated with micrococcal nuclease to reduce the level of endogenous mRNA (18) and were programmed with translatable adenovirus type 2 (Ad2) mRNA prepared during the late phase of a productive infection of human cells by Ad2. Both lysates synthesized the same set of viral proteins, including fiber proteins (Fig. 1A). The CV_1 lysate was less active (~twofold, Table 1) than the HeLa lysate. This reflects not only a difference between cell types but also the difference between their modes of growth, i.e., suspension versus monolayer. Lysates prepared from HeLa suspension cultures were generally more active translationally, but HeLa monolayer lysates were less active than CV_1 monolayer lysates. The similar pattern and level of protein synthesis with monkey cell and human cell lysates suggest that the impasse is not due to a defect in the translation apparatus of the monkey cell.

However, since infection could dramatically perturb the translational system of the cell (as has been seen after poliovirus infection [14]), the effects of infection on the activity of the cell-free lysates were determined. Lysates were prepared from abortively infected (Ad2) monkey cells and from productively infected monkey cells where the block was overcome by (i) infection with the Ad2 host range mutant hr400, which contains an altered DNA-binding protein gene, or (ii) coinfection with Ad2 plus SV40. The patterns of proteins synthesized from late Ad2 mRNA were identical whether lysates from mock-infected or abortively or productively infected cells were used (Fig. 1B). The activities of the various lysates, as determined by the amount of incorpo-

† Present address: Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA 02115.

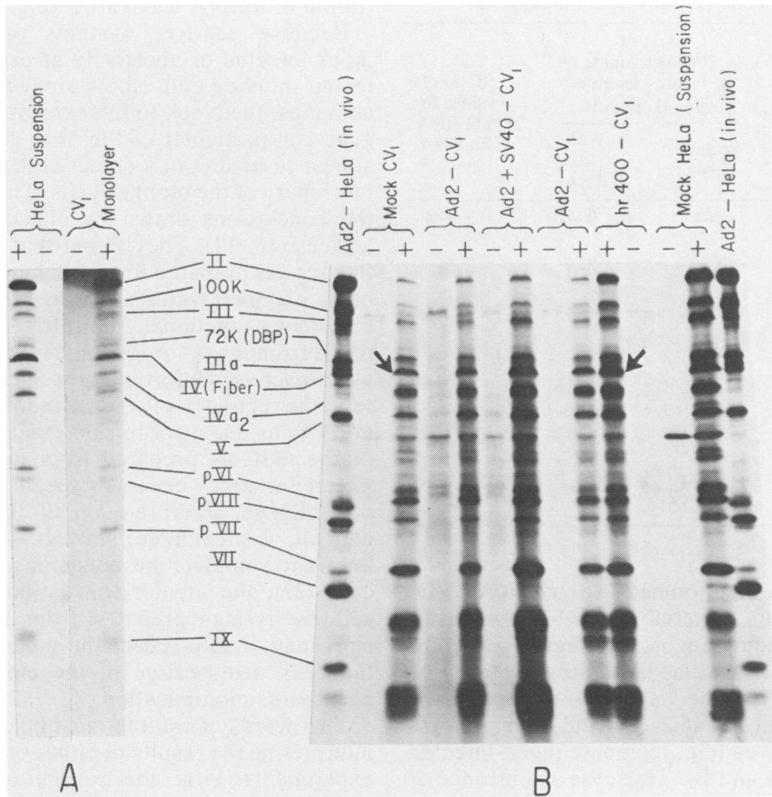

FIG. 1. Translation of Ad2 late mRNA in cell-free lysates prepared from human (HeLa) and monkey (CV_1) cells. HeLa suspension cultures at 6×10^5 cells/ml were harvested by centrifugation ($600 \times g$ for 8 min); CV_1 monolayers were harvested when they had reached 60 to 70% confluency by scraping in phosphate-buffered saline at 0°C and then by centrifugation. Monkey cells infected with 20 PFU of virus per cell were harvested 20 h postinfection, at which time the cells had reached 50 to 60% confluency. The cell pellet was washed in phosphate-buffered saline at 0°C, and all subsequent operations were done at 0°C on ice. The cell pellet was suspended in two pellet volumes of hypotonic lysis buffer (10 mM potassium acetate, pH 7.5; 10 mM HEPES [*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid], pH 7.4; 1.3 mM magnesium acetate; 2 mM dithiothreitol). After swelling for 30 min, the cells were disrupted by 20 to 40 strokes in a tight-fitting (pestle B) Dounce homogenizer. This procedure broke 90 to 95% of the cells, as determined by microscopic visualization. The nuclei were pelleted by centrifugation at $800 \times g$ for 10 min at 4°C, and the opalescent supernatant was centrifuged a second time at $10{,}000 \times g$ for 15 min at 4°C. The semiclear layer between the top lipid-like material and the pellet was divided into portions, frozen in liquid nitrogen, and stored at −70°C. Endogenous mRNA was removed by digestion with micrococcal nuclease (P-L Biochemicals, Inc.) at 20 μg/ml in 1 mM $CaCl_2$ for 5 to 10 min at 22°C. The nuclease was inactivated by the addition of EGTA [ethylene glycol-bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid] to 4 mM and was then cooled on ice. A 10-μl sample of the nuclease-treated lysate was added to the translation reaction (final volume, 20 μl), which contained 19 amino acids minus methionine at 25 μM each, 1 mM ATP, 0.2 mM GTP plus Mg^{2+}, 8 mM creatine phosphate, 0.2 mg of creatine phosphokinase per ml, 30 mM HEPES (pH 7.4), 1 mM dithiothreitol, potassium acetate at final concentrations of 110 and 94 mM for CV_1 and HeLa lysates respectively, magnesium acetate at final concentrations of 1.4 and 1.8 mM for CV_1 and HeLa lysates respectively, 25 μCi of [^{35}S]methionine at 1.1 μCi/pmol, and 7 to 14 g of total cytoplasmic RNA isolated from Ad2-infected HeLa cells 25 h postinfection. The reaction mixture was incubated at 30°C for 60 min. After adding an equal volume of sample buffer and boiling for 1 min, one-third of the reaction mixture was electrophoresed on a 17.5% sodium dodecyl sulfate-polyacrylamide gel (10). The gel was treated with Enhance (New England Nuclear Corp.) and fluorographed for several days with Kodak SB-5 X-ray film. The numbers between sections A and B denote the Ad2-specified polypeptides (DBP, DNA-binding protein; K, kilodaltons of molecular mass). Arrows indicate the fiber or IV polypeptide. The absence or presence of exogenously added Ad2 mRNA is indicated by − and +, respectively, and the sources of the lysates are shown above the tracks. Polypeptides from Ad2-infected HeLa cells labeled in vivo with [^{35}S]methionine were used as markers and are shown in the outside tracks in section B.

TABLE 1. Incorporation of [^{35}S]methionine into trichloroacetic acid-precipitable material

Cell-free lysate	Amt of [^{35}S]methionine in lysates incorporated (kcpm/μl):		Ratio of values for Ad2 RNA: no RNA
	Without RNA	With Ad2 RNA	
CV_1, mock-infected	1–4	4–10	2.5–4
CV_1, Ad2 infected	2–4	8–9	2.25
CV_1, Ad2 + SV40 infected	1.5–3	6–13	2–4.5
CV_1, hr400 infected	2–2.5	6–16	2–7
HeLa suspension	2–3	6–12	2–4
Rabbit reticulocytes	5.5	170	31

ration of [^{35}S]methionine into trichloroacetic acid-precipitable material or by band intensity on the autoradiogram, were slightly different (Table 1, Fig. 1B). Some variability in the translational activity of the lysates as well as in their ability to synthesize high-molecular-weight protein was observed (e.g., compare mock-infected CV_1 in Fig. 1A and B). Thus, the significance of the slightly lower level of synthesis of proteins in lysates from mock-infected or abortively infected cells compared with lysates from productively infected cells, seen in Fig. 1B and Table 1, is unclear since these differences were only sometimes observed.

To show that these lysates could utilize the mRNAs other than those coded by adenovirus or the host cell, the monkey cell lysates were programmed with mRNAs from rabbit globin, brome mosaic virus, and satellite tobacco necrosis virus. The lysates prepared from abortively (Ad2) and productively (Ad2 plus SV40 or hr400; data not shown for hr400) infected CV_1 cells translated each set of mRNAs with similar efficiencies to produce polypeptides of the expected size (Fig. 2).

The relative rates of translation of a population of mRNAs may be influenced by the availability of ribosomes and other limiting factors such as initiation factors (15). Thus, the translational activity of each lysate was tested with a series of mRNA concentrations for each of the mRNAs used, including those prepared during the late phase of a productive Ad2 infection of HeLa cells. The same amount of a given RNA was required for optimal translation in lysates prepared from productively and abortively infected cells. Furthermore, the pattern of proteins synthesized did not change as the concentration of mRNA was varied (e.g., Fig. 2).

Because cell-free systems prepared from mock-infected or abortively or productively infected monkey cells show similar translational activities, the block to full expression of the late genes, in particular of the fiber gene, does not appear to be due to a defect in the translational machinery of the monkey cells. This differs from the conclusions drawn by Nakajima and colleagues (16, 17). They found that whereas cell-free lysates prepared from monkey cells infected or transformed with SV40 were able to translate exogenous functional Ad2 mRNA, lysates prepared from mock- or Ad2-infected monkey cells were inactive. Unfortunately, since they did not test the activity of their lysates with other mRNAs as a control to show that the inactivity of the systems prepared from mock- or Ad2-infected monkey cells was specific for adenovirus mRNAs, interpretation of their results is difficult. For example, since SV40 infection is known to stimulate the metabolic activity of host cells (22), the greater translational activity of cell-free lysates prepared from SV40-infected cells may simply reflect the metabolic state of the cells, irrespective of the block to human adenovirus multiplication.

Two words of caution must be emphasized in interpreting the results of either of these in vitro experiments. First, the translational activity of the cell-free lysate is quite low compared with its in vivo activity. For comparison, we have contrasted the activity of our cell-free lysate prepared from CV_1 monolayers with that of a highly active rabbit reticulocyte lysate. Adenovirus late mRNA stimulates incorporation of [^{35}S]methionine into trichloroacetic acid-precipitable material about 30-fold above background in the reticulocyte system but only 2- to 4-fold in the monkey cell lysates (Table 1). However, because monkey cell lysates have larger pools of unlabeled methionine (35 μM) than the reticulocyte lysate (10 μM [21]), the latter system incorporates only about four times more picomoles of methionine per hour per microliter of lysate than does our monkey cell system (0.47 pmol versus 0.12 pmol).

The second major caution concerns the artificial nature of the in vitro system. Not only are the cellular components present in different concentrations in the lysates than in the cells, but some factor(s) or component(s) may even be missing in these lysates, such as the cytoskeletal framework to which the polysomes appear to be attached (3, 13). Furthermore, the templates for the synthesis of fiber protein may be quite different in the two systems. In monkey cells, the templates are fiber mRNA wrapped in proteins to form the ribonucleoprotein particles,

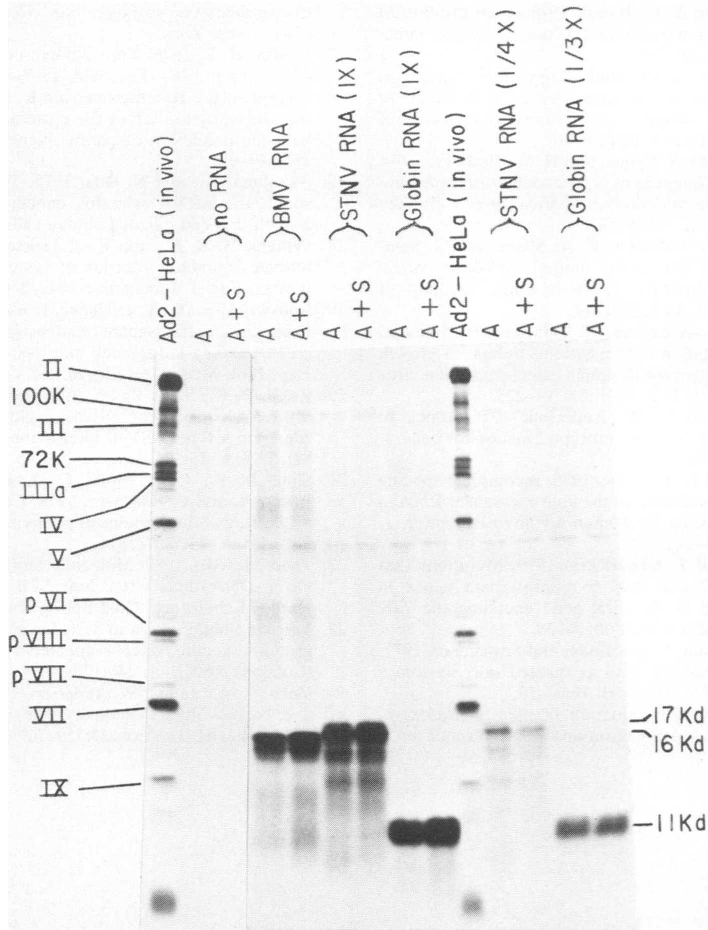

FIG. 2. Translation of several different mRNAs in lysates prepared from monkey cells infected with Ad2 (A) or Ad2 plus SV40 (A + S). Preparation and use of the cell-free translation extracts and analysis of the in vitro synthesized polypeptides are described in the legend to Fig. 1. The two cell-free translation lysates were programmed with 0.6 μg of brome mosaic virus (BMV) RNA, 1.8 (1×) or 0.45 μg (1/4×) of satellite tobacco necrosis virus (STNV) RNA, and 4 (1×) or 1.3 μg (1/3×) of rabbit globin RNA. The major polypeptides encoded by these RNAs have molecular masses of 16, 17, and 11 kilodaltons (Kd), respectively.

whereas naked RNA was used to prime fiber protein synthesis in vitro. In addition, the majority of the fiber RNAs that were isolated from infected human cells and added to the cell-free lysates did not contain the ancillary leader sequences ($x = 77$ map units, $y = 79$ map units, $z = 85$ map units; reference 4) found on most fiber mRNA synthesized in productively and abortively infected monkey cells (11).

Our current results imply that the protein-synthesizing apparatus is functional in abortively infected monkey cells. Subtle defects, if they exist, may however remain undetected until the in vitro system more closely mimics the environment present within these cells. The ability of monkey-human cell hybrids, consisting of monkey cell cytoplasm and infected human cell nuclei, to synthesize fiber protein, however, also suggests that the monkey cells' translational apparatus is competent to translate functional fiber mRNA (24).

LITERATURE CITED

1. **Anderson, K. P., and D. F. Klessig.** 1982. Synthesis of human adenovirus early RNA species is similar in productive and abortive infections of monkey and human cells. J. Virol. **42**:748–754.
2. **Celma, M. L., and E. Ehrenfeld.** 1974. Effect of poliovirus double-stranded RNA on viral and host-cell protein synthesis. Proc. Natl. Acad. Sci. U.S.A. **71**:2440–2444.
3. **Cervera, M., G. Dreyfuss, and S. Penman.** 1981. Messenger RNA is translated when associated with the cytoskeletal framework in normal and VSV-infected HeLa cells. Cell **123**:113–120.
4. **Chow, L. T., and T. R. Broker.** 1978. The spliced structures of adenovirus 2 fiber message and the other late mRNAs. Cell **15**:497–510.

5. **Farber, M. S., and S. G. Baum.** 1978. Transcription of adenovirus RNA in permissive and nonpermissive infections. J. Virol. **27**:136–148.
6. **Feldman, L. A., J. S. Butel, and F. Rapp.** 1966. Interaction of a simian papovavirus and adenoviruses. I. Induction of adenovirus tumor antigen during abortive infection of simian cells. J. Bacteriol. **91**:813–818.
7. **Friedman, M. P., M. J. Lyons, and H. S. Ginsberg.** 1970. Biochemical consequences of type 2 adenovirus and simian virus 40 double infections of African green monkey kidney cells. J. Virol. **5**:586–597.
8. **Grodzicker, T., C. Anderson, P. A. Sharp, and J. Sambrook.** 1974. Conditional lethal mutants of adenovirus 2-simian virus 40 hybrids. I. Host range mutants of Ad2^{+}ND1. J. Virol. **13**:1237–1244.
9. **Hashimoto, K., K. Nakajima, K. Oda, and H. Shimojo.** 1973. Complementation of translational defect for growth of human adenovirus type in simian cells by a simian virus 40-induced factor. J. Mol. Biol. **81**:201–223.
10. **Klessig, D. F., and C. W. Anderson.** 1975. Block to multiplication of adenovirus serotype 2 in monkey cells. J. Virol. **16**:1650–1688.
11. **Klessig, D. F., and L. T. Chow.** 1980. Incomplete splicing and deficient accumulation of the fiber messenger RNA in monkey cells infected by human adenovirus type 2. J. Mol. Biol. **139**:221–242.
12. **Klessig, D. F., and T. Grodzicker.** 1979. Mutations that allow human Ad2 and Ad5 to express late genes in monkey cells map in the viral gene encoding the 72K DNA binding protein. Cell **17**:957–966.
13. **Lenk, R., L. Ransom, Y. Kaufman, and S. Penman.** 1977. A cytoskeletal structure with associated polyribosomes obtained from HeLa cells. Cell **10**:67–78.
14. **Levintow, L.** 1974. Reproduction of picornaviruses, p. 109–170. *In* H. Fraenkel-Conrat and R. R. Wagner (ed.), Comprehensive virology, vol. 2. Plenum Publishing Corp., New York.
15. **Lodish, H. F.** 1976. Translational control of protein synthesis. Annu. Rev. Biochem. **45**:39–72.
16. **Nakajima, K., H. Ishitsuka, and K. Oda.** 1974. An SV40-induced initiation factor for protein synthesis concerned with the regulation of permissiveness. Nature (London) **252**:649–653.
17. **Nakajima, K., and K. Oda.** 1975. The alteration of ribosomes for mRNA selection concerned with adenovirus growth in SV40 infected simian cells. Virology **67**:85–93.
18. **Pelham, H. R. B., and R. J. Jackson.** 1967. An efficient mRNA-dependent translation system from reticulocyte lysates. Eur. J. Biochem. **67**:247–256.
19. **Rabson, A. S., G. T. O'Conor, I. K. Berezesky, and F. J. Paul.** 1964. Enhancement of adenovirus growth in African green monkey kidney cell cultures by SV40. Proc. Soc. Exp. Biol. Med. **116**:187–190.
20. **Reich, P. R., S. G. Baum, J. A. Rose, W. P. Rowe, and S. M. Weissman.** 1966. Nucleic acid homology studies of adenovirus type 7-SV40 interactions. Proc. Natl. Acad. Sci. U.S.A. **55**:336–341.
21. **Shih, D. S., C. T. Shih, O. Kew, M. Pollansch, R. Rueckert, and P. Kaesberg.** 1978. Cell-free synthesis and processing of the proteins of poliovirus. Proc. Natl. Acad. Sci. U.S.A. **75**:5807–5811.
22. **Tooze, J. (ed.).** 1980. Molecular biology of tumor viruses; Part 2, DNA tumor virus, 2nd ed., p. 127–130. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
23. **Van der Vliet, P. C., and A. J. Levine.** 1973. DNA binding proteins specific for cells infected by adenovirus. Nature (London) New Biol. **246**:170–173.
24. **Zorn, G. A., and C. W. Anderson.** 1981. Adenovirus type 2 expresses fiber in monkey-human hybrids and reconstructed cells. J. Virol. **37**:759–769.