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Adenovirus E1B Proteins Are Required for Accumulation of Late Viral mRNA and for Effects on Cellular mRNA Translation and Transport

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Late in adenovirus infection, large amounts of viral mRNA accumulate while cell mRNA transport and translation decrease. Viruses deleted in the E1B region of type 5 adenovirus do not produce the same outcome: (i) viral mRNA synthesis by the mutants is normal, delivery to the cytoplasm is 50 to 75% of normal, but steady-state levels of viral mRNA are decreased 10-fold; (ii) cell mRNA synthesis and transport continue normally in the mutant virus-infected cell; and (iii) translation of preexisting cell mRNA which is disrupted in wild-type infection remains normal in mutant-virus-infected cells. Thus E1B proteins are required for accumulation of virus mRNA and for induction of the failure of host cell mRNA transport and translation. If a single function is involved, by inference the transport and some aspect of translation of mRNAs could be linked.

Late in the course of adenovirus infection, host protein synthesis is replaced almost entirely by virus protein synthesis. This phenomenon has received considerable study (2–5, 15, 27). It is now established that preexisting host mRNA is not destroyed, but is no longer translated (2, 15). Furthermore, it is now known that VA RNA is required to maintain late viral translation (27) and apparently does so by preventing the excess phosphorylation of the protein initiation factor E1F-2 (20). How this restitution of active EIF-2 specifically leads to viral mRNA translation is not understood. Without VA late in infection, however, neither viral nor host mRNA is translated (2, 27).

In addition to the translation changes that occur late in infection, new cell mRNA fails to accumulate in the cytoplasm despite continued nucleus synthesis (2, 5). Whether this interruption is related to the effect on translation of cell mRNA is unknown, but both events do occur.

In studying two type 5 adenovirus mutants containing deletions in the E1B region (H5*dl*118 and H5*dl*163; referred to as *dl*118 and *dl*163), it was earlier noted that DNA replication occurred normally, and, based on [^{3}H]uridine incorporation, late viral RNA synthesis began normally, but late viral protein synthesis was decreased (3). In addition, total host protein synthesis did not appear to be deranged.

We have further characterized host and viral mRNA metabolism in cells infected with these mutant viruses compared with that in the wild-type virus (*sub*309). We find further quantitative evidence that transcription of late viral sequences is normal and that delivery of late viral mRNA to the cytoplasm is near normal, but that the total late viral mRNA is decreased 10-fold. This viral phenotype is associated with a dramatic reversal of the effects of wild-type infection on host cell mRNA functions. Not only does host cell mRNA synthesis continue, but entrance into the cytoplasm is normal and host cell translation is quantitatively maintained. Thus it appears that the two major effects of late viral infection, high accumulation and near exclusive translation of viral mRNA and low accumulation and translation of cell mRNA are reciprocally linked.

MATERIALS AND METHODS

Cells and viruses. Monolayer cultures of human HeLa and KB cell line 18 (a KB cell line that constitutively expresses the bacterial XGPRT gene and the adenovirus 2 E1B gene [4]) cells were grown in Dulbecco modified Eagle medium supplemented with 10% calf serum. Suspension cultures of human KB and HeLa cells were grown in modified minimal essential medium supplemented with 10% human serum or 10% fetal calf serum, respectively.

H5*sub*309, a type 5 adenovirus with wild-type properties (13), was provided by T. Shenk and was propagated in either KB or HeLa cell suspension cultures. The deletion mutants (*dl*118 and *dl*163) have been described previously (3) and were propagated in KB cell line 18 cell monolayers.

RNA analysis. The isolation of both cytoplasmic and nuclear RNA from uninfected and virus-infected HeLa cell monolayers and suspension cultures has been previously described (3, 18). For the continuous [^{3}H]uridine labeling experiment (Fig. 3), replicate monolayer cultures of HeLa cells were infected with *dl*118 or *sub*309 at 20 PFU per cell, and at 20 h postinfection 200 μCi of [^{3}H]uridine per ml was added with unlabeled uridine (14 μM), which prevented the depletion of uridine during the course of the experiment. Polyadenylated [poly(A)$^+$] cytoplasmic and nuclear RNAs were isolated by chromatography on oligo(dT)-cellulose (type 3; Collaborative Research, Inc.). Virus-specific L3 and L5 mRNA species were identified by electrophoretically fractionating denatured RNAs on agarose-formaldehyde gels (10), transferring the RNAs to nitrocellulose filters (28), and hybridizing them with nick-translated, ^{32}P-labeled (21) DNA probes.

Transcription in isolated nuclei and dot hybridization. The extraction and in vitro labeling of nuclei from *dl*118, *sub*309, or uninfected HeLa cell monolayers infected at 20 PFU per cell at 20 and 35 h postinfection were performed as described by Hofer and Darnell (12) and Weber et al. (30). In the transcription assay we used approximately 10^8 nuclei and

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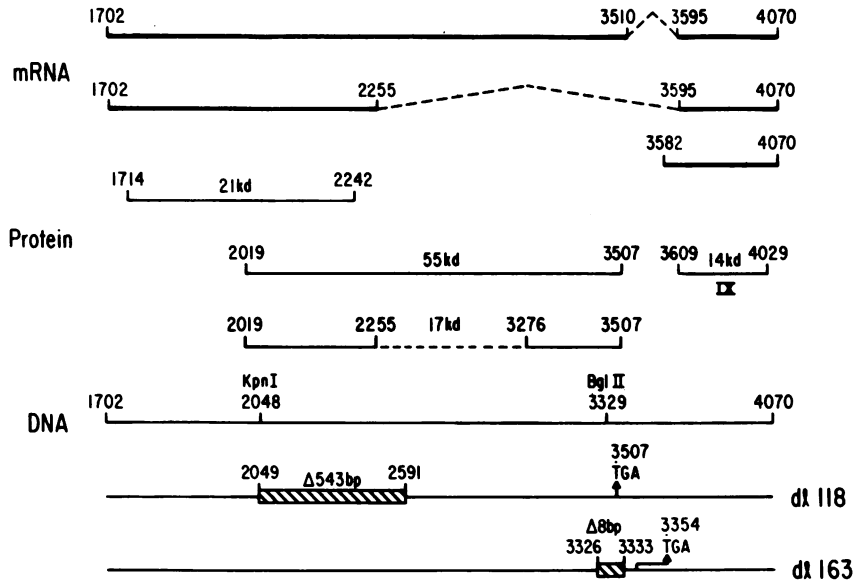

FIG. 1. Nucleotide sequences of the deletions present in the E1B region of *dl*118 and *dl*163. The sequencing strategy used to characterize the deletion present in both viruses has been previously described (3). Hatched boxes show the deleted (Δ) DNA sequence in each mutant. Dashed lines represent sequences removed during the processing of the 22S and 13S mRNAs, and the 17-kDa protein. The nucleotide positions of the splice junctions and 5′ and 3′ termini are from Bos et al. (6).

200 μCi of [^{32}P]UTP (3,000 μCi/mmol), which were incubated for 15 min at 30°C. Nuclear RNA was isolated by the guanidinium isothiocyanate method of Ullrich et al. (29) and before hybridization was broken by treatment with 0.2 N NaOH for 15 min on ice (18). The preparation of nitrocellulose filters containing "dots" of denatured DNA was done as described by Kafatos et al. (14).

In vitro translation, immunoprecipitation, and SDS-polyacrylamide gel electrophoresis. Poly(A)$^+$ cytoplasmic RNA was isolated from mock-, *sub*309-, or *dl*118-infected HeLa cell monolayers at 20 and 35 h postinfection. Approximately 1 μg of RNA from each sample (see Fig. 4) was added to a rabbit reticulocyte extract (Promega Biotech). Translation was carried out for 2 h at 30°C, and the samples were boiled in sample buffer (2% sodium dodecyl sulfate [SDS], 0.01% bromophenol blue, 10% glycerol, 5% β-mercaptoethanol) for 3 min. The samples were fractionated on SDS-polyacrylamide gels containing 17.5% polyacrylamide and 0.7% bisacrylamide (16). After electrophoresis, the gels were fixed with 50% (vol/vol) methanol–10% acetic acid, enhanced (En^{3}Hance; New England Nuclear Corp.), dried, and exposed to X-ray film.

The proteins of uninfected, mutant-infected, or *sub*309-infected HeLa cells were labeled with [^{35}S]methionine (10 μCi/ml) in the presence of 5% of the usual concentration of methionine at 19 and 35 h postinfection. At the end of the labeling period, cells were harvested, and samples containing equal numbers of trichloroacetic acid-precipitable counts were processed by immunoprecipitation with polyclonal antiserum against viral and cellular polypeptides as previously described (3, 19). Anti-keratin antiserum was a gift from H. Green, and rabbit anti-vimentin antiserum was a gift from R. Hynes.

RESULTS

To determine whether the absence of wild-type E1B proteins in infected cells affects the transcription, cytoplasmic accumulation, or translation of both viral and cellular mRNAs, these parameters were compared in uninfected cells and cells infected with two E1B mutants or wild-type virus. The two E1B mutant viruses used in the experiments to be described are *dl*118 and *dl*163, whose genomic mutations are illustrated in Fig. 1. The wild-type virus used in the experiments is *sub*309, the parent virus used for the construction of *dl*118 and *dl*163, which is phenotypically similar to the original prototype type 5 adenovirus (13). A correlation between the E1B polypeptides affected by the mutations present in both of the E1B-mutant viruses and their phenotypic characteristics to be described will be presented in the Discussion.

Virus- and host-specific nuclear RNA synthesis late in infection. We previously found by using a 10-min [^{3}H]uridine incorporation into whole cells, that the rates of transcription of the late viral regions L3 (containing the hexon coding exon from 50.1 to 60 map units [m.u.]) and L5 (containing the fiber exon from 84 to 91.5 m.u.) appeared to be comparable in *dl*118 and wild-type virus-infected KB cells (3). To extend this analysis to the entire late transcription unit and to quantitatively examine transcription of cellular genes in infected cells, labeled nascent RNA samples were prepared from isolated HeLa cell nuclei and hybridized to dots of DNA on nitrocellulose (14). Since RNA chain initiation in isolated nuclei is inefficient, but chain elongation of previously initiated RNA polymerase II molecules occurs faithfully, this assay is perhaps the best test of differential rates of RNA synthesis (reviewed in reference 9). In such experiments, the same amount of total labeled RNA (representing approximately equivalent numbers of nuclei), from each nuclear sample was used in the hybridization assay so that differential rates of RNA synthesis could be measured. The DNA dots included regions of the adenovirus late transcriptional unit from about 30 to 93 m.u., the E1A transcription unit, and the protein 9 transcription unit; actin and tubulin cDNAs served as controls for cellular genes. In this analysis (Fig. 2), the transcription rates for all the viral transcription units described were similar in *dl*118- and *sub*309-infected HeLa cells (transcription was also similar in cells infected with *dl*163 [data not shown]). Each dot from the major late

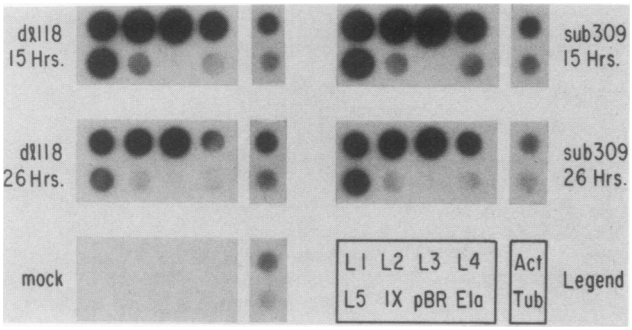

FIG. 2. Dot hybridization of RNA labeled in nucleic isolated from mock-, *sub*309, and *dl*118-infected HeLa cells. HeLa cell monolayers were infected with 20 PFU of the appropriate virus per cell, and at the times indicated nuclear RNA was labeled in vitro with [^{32}P]UTP as described in Materials and Methods, and 2.5 × 10^6 CPM was hybridized to a dot containing 7 μg of L1 (31.5 to 37.3 m.u.), L2 (41 to 50.1 m.u.), L3 (50.1 to 61 m.u.), L4 (72.8 to 80 m.u.), L5 (84 to 93 m.u.), E1A (0.0 to 4.5 m.u.), actin, tubulin, and pBR322 DNA.

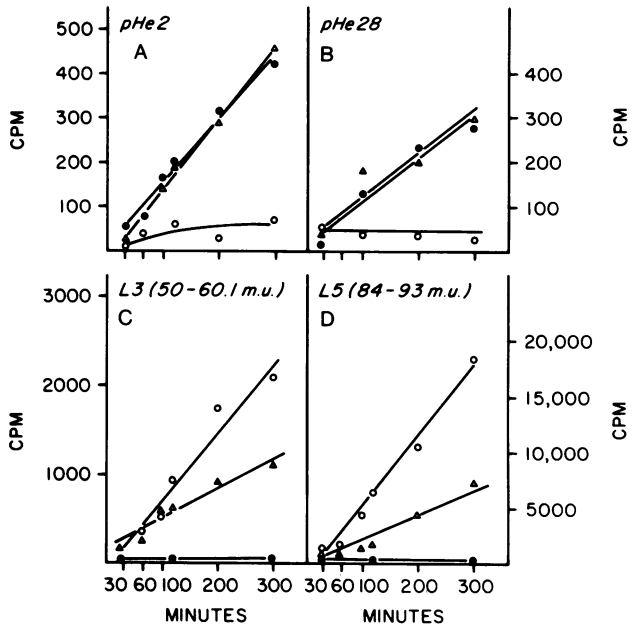

FIG. 3. Cytoplasmic accumulation of specific HeLa cell mRNAs and late adenovirus mRNAs in mock- (●), *dl*118- (△), or *sub*309- (○) infected HeLa cells. HeLa cell monolayers were infected with 20 PFU of the appropriate virus per cell, and at 20 h postinfection [^{3}H]uridine was added to a concentration of 200 μCi per ml with cold uridine (14 μM). At the times indicated, 2 × 10^7 cells were harvested, poly(A)$^+$ cytoplasmic RNA was prepared, and the equivalent of 5 × 10^6 cells labeled RNA were hybridized to filters bearing plasmid DNAs (5 μg) specific for the HeLa cell mRNAs pHe7 and pHe28 (2) or adenovirus late mRNAs L3 (50.1 to 61 m.u.) and L5 (84 to 93 m.u.).

transcription unit gave approximately an equal signal when traced in a densitometer (Table 1) and normalized for the lengths of the probe. (The tracings were carried out for viral dots on films exposed for less time than in Fig. 2 and for cell genes in films for Fig. 2. The use of preflashed film has been shown to give linear signals for both time of exposure and RNA input [D. F. Clayton and J. E. Darnell, submitted for publication].) These results indicated equimolar transcription across the entire transcription unit for both *sub*309 and *dl*118 viruses. The transcription rates for both actin and tubulin were similar (within a factor of 2) for all the nuclear samples. If anything, transcription of both actin and tubulin was slightly increased at both 15 and 26 h after infection in cells infected with either *dl*118 or *sub*309. Increased transcription of α-tubulin early in adenovirus infection has been reported (26). These transcriptional results on cell-specific genes are in accord with the studies of Babich et al. (2) and Beltz and Flint (5) for wild-type virus and suggest that the lack of E1B proteins in cells infected by *dl*118 or *dl*163 does not alter viral or cellular transcription.

Newly synthesized viral mRNAs enter the cytoplasm of *dl*118-infected cells. Beltz and Flint (5) reported that the majority of labeled RNA that reaches the cytoplasm and the polyribosomes late in infection of HeLa cells is complementary to viral DNA. Since nuclear transcription was not affected by altered E1B gene expression, we determined the time course of cytoplasmic appearance of labeled RNA complementary to the viral L3 and L5 late transcription regions in both *sub*309- and *dl*118-infected cells. The emergence of new RNA in the cytoplasm was tested by labeling cells with [^{3}H]uridine, preparing samples of poly(A)$^+$ RNA at various times, and hybridizing the labeled RNA to specific cloned viral DNA sequences. The late viral sequences, including hexon (L3; Fig. 3C) and fiber (L5; Fig. 3D) mRNAs, appeared in the cytoplasm of both cells infected with *dl*118 and *sub*309, but the rate of appearance in *dl*118-infected cells was reduced by 30 to 50% compared with that in cells infected with *sub*309. In a second experiment of this type, the rates observed in *dl*118-infected cells were 50 to 70% those of *sub*309-infected cells (data not shown). Also, this lack of efficient initial accumulation of late viral mRNAs in *dl*118-infected cells persisted through 35 h after infection (data not shown).

TABLE 1. Relative transcription rates of viral and cellular genes in uninfected and *dl*118- and *sub*309-infected HeLa cells[a]

Infectious agent, time (h)	Relative rates for plasmid DNAs:								
	L1	L2	L3	L4	L5	E1A	pIX	β-Actin	α-Tubulin
*sub*309, 15	1.01	1.0	1.0	0.99	0.95	1.0	1.0	1.2	1.1
*dl*118, 15	1.09	1.03	1.07	1.1	0.96	1.2	0.8	1.15	1.08
*sub*309, 26	0.95	1.0	1.0	0.94	0.94	1.0	1.0	1.2	1.05
*dl*118, 26	0.91	0.95	0.98	0.87	0.87	1.15	1.2	1.25	1.3
Mock, 26	—[b]	—	—	—	—	—	—	1.0	1.0

[a] For comparison of transcription rates, the data in Fig. 2 and additional autoradiograms of lighter exposure were analyzed by densitometric scanning. The numbers entered are arbitrary units. The major late transcription unit signal for L3 in *sub*309-infected cells was taken as 1.0, and numbers for other regions were corrected for the lengths of the probes and normalized to L3. The viral E1A and IX transcriptional signals are relative to *sub*309-infected HeLa cells, and the cellular actin and tubulin signals are relative to mock-infected HeLa cells.

[b] —, No significant signal over background.

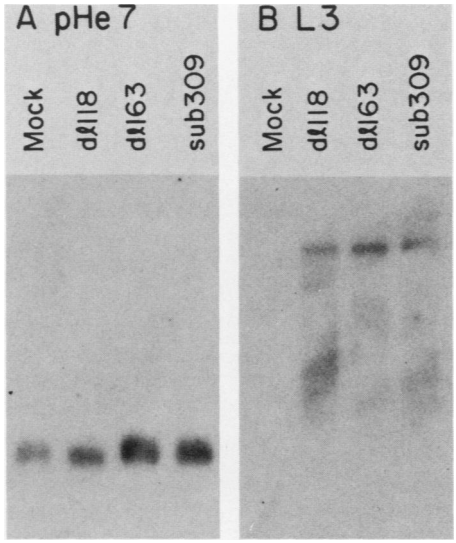

FIG. 4. Analysis of viral and host cell nuclear mRNA species present at 20 h after infection of HeLa cells with *dl*118, *dl*163, or *sub*309 or uninfected HeLa cells. Poly(A)$^+$ nuclear RNAs were isolated from HeLa cells infected with 20 PFU of the appropriate virus per cell. (A) Denatured RNA (5 μg) and (B) denatured RNA (0.1 μg) were fractionated on 0.8% agarose gels containing 2.2 M formaldehyde as described in Materials and Methods. After transfer to nitrocellulose filters, RNAs were hybridized with nick-translated L3 (50 to 60.1 m.u.; panel B) or pHe7 (panel A) DNA. The L3-specific mRNAs produced were 3.96 and 3.24 kilobases (kb) in size; the pHe7 mRNA was 1.05 kb.

These data suggest either that viral nuclear transcripts are not processed efficiently in *dl*118-infected cell nuclei, or that E1B proteins may either catalyze the transport of viral mRNAs to the cytoplasm or stabilize the viral RNAs once transported to the cytoplasm.

Steady-state amounts of viral mRNAs in the nucleus and cytoplasm of infected cells. To determine whether the small changes in the cytoplasmic appearance of labeled mRNA was accompanied by any change in nuclear RNA composition, Northern blots of nuclear poly(A)$^+$ RNA were carried out. There was about the same amount of nuclear RNA complementary to the viral sequences in the nuclei of *dl*118-, *dl*163-, and *sub*309-infected cells (Fig. 4). All of the nuclear RNA detected was the same size as cytoplasmic RNA, indicating that processing of the various RNAs was normal. This last conclusion is based on the assumption that the RNA isolated was in fact in the nucleus within the cell and not a cytoplasmic contamination. The conclusion is reasonable on the basis of several earlier observations. All nuclear poly(A)$^+$ molecules recovered from nuclei prepared in the manner used for these experiments contain only the full-length 250-nucleotide poly(A), while the poly(A) associated with cytoplasmic RNA at steady state is much shorter. Therefore, it appears that the cell fractionation procedures does in fact result in preparation of nuclear RNA uncontaminated with cytoplasmic mRNA to any significant extent (23–25). Thus, Northern blot experiments (Fig. 4) indicate that there is not a piling-up in the nucleus of unprocessed or untransported mRNA-sized molecules.

The more revealing findings on steady-state concentrations of RNA were made with cytoplasmic RNA. Northern blot analysis for specific viral mRNA sequences showed that the abundance of viral mRNAs 20 h after infection with *dl*118 was greatly reduced when compared with that after *sub*309 infection (Fig. 5C and D), a result that had been previously observed (3). Densitometric tracing of several different northern blot experiments for L3 and L5 mRNAs at 16 to 20 h after infection showed a decrease of between 5- and 10-fold in mutant virus-infected cells. At 35 h postinfection the amount of viral mRNA in *dl*118-infected cells had increased, although there was still much more viral mRNA in the cytoplasm of cells infected with *sub*309 (Fig. 5C and D). This experiment and that shown in Fig. 3 show that the steady-state levels of the L3 and L5 mRNAs in *dl*118-infected HeLa cells was reduced by a factor of at least 5 to 10 relative to levels for infection with wild-type virus, while the rate of initial apperance of labeled mRNA was decreased less than a factor of 2. Thus, a major effect of E1B polypeptides is to allow accumulation of late viral mRNA, presumably by increasing stability and perhaps also increasing the transport of late adenovirus mRNAs.

Newly synthesized host RNAs enter the cytoplasm of *dl*118-infected cells. At late times after infection with wild-type adenovirus, several points have been established about host cell mRNA metabolism. First, although the accumulation of newly made specific host cell mRNA in the cytoplasm is decreased, the preexisting copies of the same cellular mRNAs remain, leaving the steady-state level of host

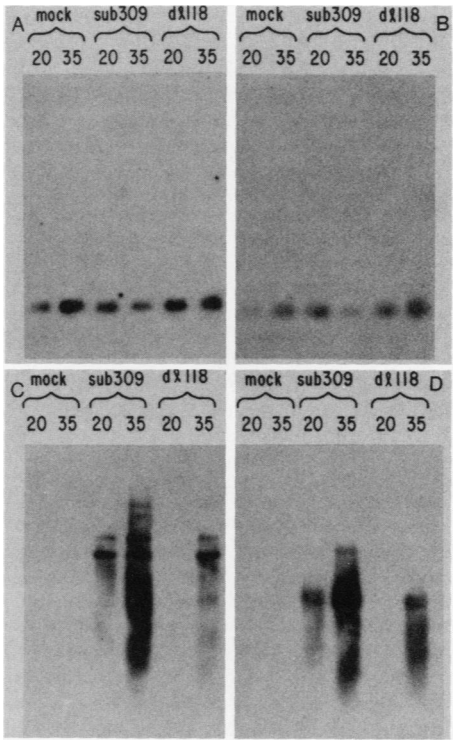

FIG. 5. Analysis of viral and host cell cytoplasmic mRNA species present in mock-, *dl*118-, or *sub*309-infected HeLa cells. Poly(A)$^+$ cytoplamic RNA was isolated from HeLa cells at 20 and 35 h after infection with 20 PFU per cell of *dl*118 or *sub*309. (A and B) Denatured RNA (5 μg) and (C and D) denatured RNA (0.1 μg) were processed as described in Materials and Methods. Filter-bound RNAs were hybridized with nick-translated pHe7 (panel A), pHe28 (panel B), L3 (50 to 60.1 m.u.; panel C) or L5 (84 to 93 m.u.; panel D) DNA. The L3-specific mRNAs produced by *sub*309 and *dl*118 were 3.96 and 3.24 kb in size, and the L5-specific mRNAs were 1.93 and 1.8 kb. The pHe7 and pHe28 mRNAs were 1.05 and 0.62 kb, respectively.

mRNA unchanged. However, the host cell mRNA is not efficiently translated late in infection (2, 27). We next examined each of these points in uninfected HeLa cells and cells infected with *dl*118 or *sub*309 virus. The cytoplasmic accumulation of cellular mRNA species was measured as described above (Fig. 3). In uninfected cells or cells infected with *dl*118, labeled mRNAs complementary to chicken actin cDNA (not shown) and two unidentified human cDNAs (pHe7 and pHe28 [2]) accumulated at approximately linear rates for the labeling period shown (Fig. 3A and B). In contrast, in cells that were infected with *sub*309, very little labeled cellular mRNA appeared in the cytoplasm. Furthermore, all nuclear RNA detected was the same size as cytoplasmic RNA, indicating that nuclear processsing was normal (Fig. 4A), and there was no evidence to indicate that untransported pHe7 mRNA piled up in the nuclei of *sub*309-infected cells. These findings suggest that the E1b proteins either are directly involved in the prevention of cellular mRNA transport, or indirectly reduce cell mRNA transport by promoting the more efficient transport of cytoplasmic stabilization of viral RNAs.

However, as has been found earlier (2, 15), even though labeled cell mRNA accumulation was decreased by infection with *sub*309, the total amount of steady-state mRNA complementary to specific cellular probes remained about the same as for control cells. By 35 h after infection with *sub*309, there was still only about a twofold decrease in the cellular mRNAs (Fig. 5A and B). Likewise, infection with *dl*118 virus did not decrease the level of cellular mRNAs by 20 or even by 35 h after infection. With the above conclusions on mRNA synthesis and accumulation established (*sub*309 stops all mRNA accumulation in favor of high levels of viral mRNA; *dl*118 allows normal levels of cell mRNA accumulation but greatly decreases viral mRNA accumulation), we turned to comparing translation of specific mRNAs in wild-type- and *dl*118-infected cells.

Inability to synthesize late viral antigens is not due to a translational block. First it seemed possible that E1B protein(s) could play a direct role in viral mRNA translation without which accumulation of viral mRNAs might be faulty. Therefore the rate of hexon synthesis was compared in *dl*118-, *dl*163-, and *sub*309-infected cells. Antibody precipitation of the hexon protein followed by gel analysis of the antibody precipitates was carried out on extracts of [^{35}S]-methionine-labeled cells. The *dl*118-infected cells synthesized between 5- and 10-fold less hexon protein than did *sub*309-infected cells (see Fig. 7). This decrease was commensurate with the decrease of viral mRNA concentration noted in Fig. 5, indicating no failure of translation of viral products in *dl*118-infected cells.

The distribution in polysomes of one of the late mRNAs in *dl*118-infected cells was then determined by a Northern blot analysis of fractions of different-sized polyribosomes. The L3 mRNA, which includes the hexon mRNA, was found to be present in polysomes averaging five to eight ribosomes in *dl*118-infected cells (data not shown [3]). This signifies normal polysome loading. These experiments suggest that there is no direct participation of the E1B proteins in initiating or completing translation of late viral mRNAs, but that translation of these mRNAs in *dl*118- and wild-type virus-infected cells occurs in proportion to their cytoplasmic content.

Translation of specific cell proteins in infected cells. In addition to examining viral translation, we reexamined host protein synthesis in virus-infected cells, again including an analysis of synthesis rates for specific proteins. We previously reported that host protein synthesis was still detected by ^{35}S incorporation into cells at 20 and 35 h after *dl*118 infection (3), indicating that viral protein synthesis had not supplanted host cell translation, as occurs in *sub*309-infected cells. A similar result was also obtained with isolated mRNA translated in vitro (Fig. 6), signifying that the viral mRNA was not dominant in concentration in the *dl*118-infected cells. Just as was the case when [^{35}S]methionine was used to pulse label *dl*118-infected cells, there was a general decrease in virus-specific protein bands on polyacrylamide gels after the in vitro reaction (Fig. 6, last four lanes).

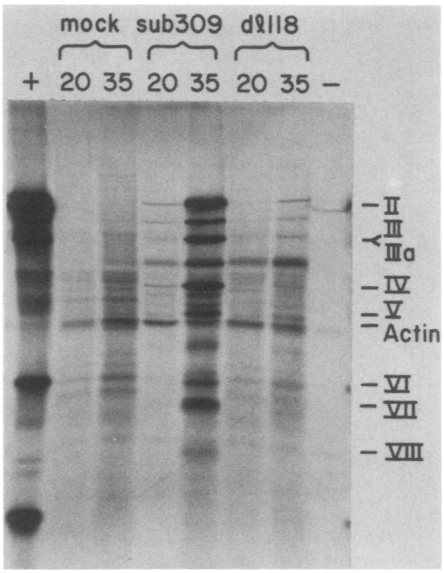

FIG. 6. SDS-polyacrylamide gel autoradiogram of ^{35}S-labeled in vitro translation protein products with uninfected, *dl*118-, or *sub*309-infected HeLa cell cytoplasmic poly(A)$^+$ RNA. HeLa cell monolayers were infected with 20 PFU of *dl*118 or sub309 per cell, and at 20 and 35 h after infection cytoplasmic poly(A)$^+$ RNA was isolated as described in the text. A portion (1 μg) of each RNA sample was translated in vitro by a rabbit reticulocyte lysate system with [^{35}S]methionine as the label. All proteins extracted were prepared for gel electrophoresis and autoradiography as described in the text. The lane labeled (–) shows the translation products obtained in the absence of exogenous RNA. The lane labeled (+) represents the translation products obtained with 1 μg of brome mosaic virus RNA in the translation reaction; the sizes of the major viral proteins synthesized were 110, 97, 35, and 20 kDa.

To determine whether the interruption of host cell protein synthesis by *sub*309 occurs for specific proteins, cells were labeled with [^{35}S]methionine for 2 h at two different times after infection, and labeled protein extracts were exposed to antiserum against keratin (65 kilodaltons [kDa]) and vimentin (58 kDa) (Fig. 7). The formation of both these proteins was inhibited during wild-type virus infection (5-fold at 20 h and 8- to 10-fold at 35 h), but little inhibition occurred after *dl*118 or *dl*163 infection (no more than 2-fold). This again emphasizes that in E1B-infected cells, in which transport of cell mRNA still occurred, translation of these nascent cellular mRNAs can still occur. However, the opposite is true in wild-type virus-infected cells: no cell mRNA transport occurred, and interruption of cellular translation of preexisting mRNAs was in force.

DISCUSSION

In this work we have concentrated on the possible relationship of two effects that are imposed on host cells late in

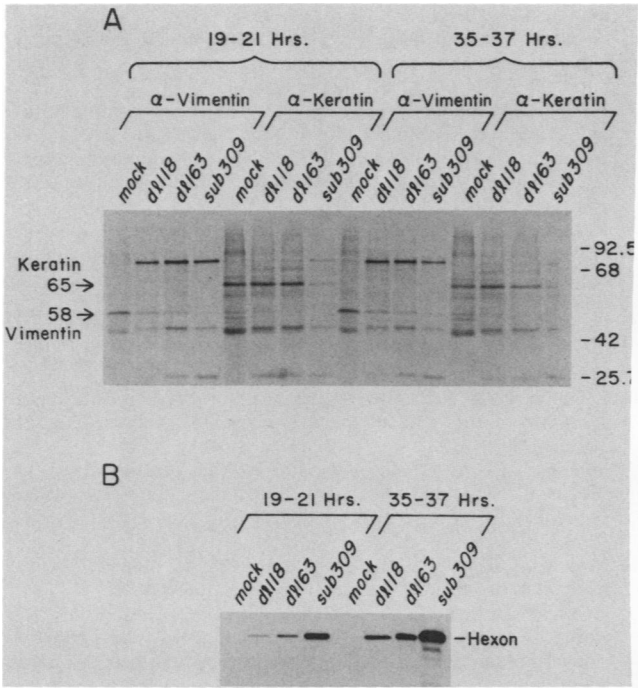

FIG. 7. Immunoprecipitation of hexon, vimentin, and keratin polypeptides in mock- and virus-infected HeLa cells. HeLa cell monolayers were infected with 20 PFU of *dl*118, *dl*163, or *sub*309 per cell, and at the times indicated they were labeled with [^{35}S]methionine (10 μCi/ml). Whole-cell extracts were prepared, and virus-specific hexon (panel B), cellular vimentin (panel A), and cellular keratin (panel A) polypeptides were detected with polyclonal serum as described in Materials and Methods. The samples were analyzed by SDS-polyacrylamide gel electrophoresis and fluorography.

wild-type adenovirus infection: a decrease in cell mRNA translation and a decrease in cytoplasmic appearance of newly made cellular mRNAs, even though nuclear synthesis of cellular sequences persists (2, 5). Mutants of type 5 adenovirus that do not make normal E1B proteins replicate their DNA normally and undergo transcription of late RNA in the nucleus at a rate comparable to that of wild-type viruses (Fig. 2), but do not accumulate viral mRNAs normally (Fig. 3). These same mutants do not stop cellular mRNA translation (Fig. 5; also see reference 3) and do not prevent appearance of newly labeled cellular mRNAs in the cytoplasm (Fig. 3). These results can be understood by assuming one of two possible roles for the E1B protein(s): (i) the E1B protein(s) inactivates a cellular function that interferes with established translation, and this same function plays a role in transporting new cellular mRNAs into the cytoplasm; and (ii) the E1B protein plays a role in the efficient transport or stabilization, or both, of late adenovirus mRNAs. The first proposal would imply that some adenovirus function must arise to fulfill the role of inactivated cell function for late viral mRNAs. In the latter proposal, interruption by wild-type virus of host protein synthesis and mRNA transport could be secondary to the effectiveness of the late adenovirus mRNA transport and translation system which requires the E1B proteins.

In this work we have not yet concentrated on the exact polypeptides responsible for the wild-type virus phenotype. However, some conclusions seem valid by reference to the genomic structures of the E1B mutant viruses used in the present studies. Both the mutant viruses used in these experiments contain deletions that prevent the formation of a spliced 22S mRNA that encodes the 55-kDa polypeptide (Fig. 1). In addition, a newly discovered 17-kDa protein is encoded within E1B (1), and both the *dl*118 and *dl*163 mutations would prevent its formation. Therefore, either the major 55-kDa or minor 17-kDa product would be candidates for producing the late adenovirus phenotype. In addition, the *dl*118 deletion also prevents production of a complete 13S mRNA that nomally encodes the 21-kDa polypeptide, but the *dl*163 mutation allows normal 21-kDa polypeptide formation. Since *dl*163 has a similar phenotype to that of *dl*118, we can conclude that the 21-kDa protein alone will not bring on the late phenotype. Therefore, cells infected with *dl*118 and *dl*163 which are defective in encoding 55- and 17-kDa proteins both show the major results described here: a failure to accumulate viral mRNAs while still accumulating cell mRNAs with a related continuation in cell mRNA translation.

One additional point about the effects of E1B proteins is pertinent. Monoclonal antibodies raised against the E1B-encoded 55-kDa protein precipitate an apparent complex between the E1B 55-kDa protein and a protein encoded in the E4 region (34 kDa) of the adenovirus genome (22). Moreover, cell lines which contain viral E1B gene sequences and constitutively synthesize the 55-kDa polypeptide do so without apparent ill effect (4, 11). Since viruses containing mutations affecting E4 proteins also allow continued host cell protein synthesis (8, 22), the activity which is ascribed to E1B proteins in productive infection may well occur only when they are complexed to E4 protein.

Experiments with another mutant virus that is known to affect translation in infected cells must be considered in the context of the two proposed possibilities for E1B interruption of host functions. Cells infected with viruses lacking the sequences encoding the 160-nucleotide VA1 RNA do not translate viral mRNA efficiently, although they accumulate viral mRNAs to an extent similar to that of cells infected by wild-type virus (27). In other experiments (2), an inhibition of cellular translation during infection with the VA$^-$ mutant has been found. We have also found (A. Babich, J. R. Nevins, and J. E. Darnell, unpublished data) that new cell mRNA accumulation is still inhibited in the VA1$^-$ mutant. Thus in the case of VA mutants as well as E1B mutants, one correlation holds: when cell mRNA translation is decreased, viral RNA accumulation is high and cell mRNA accumulation is low.

If successful translation were always coupled with efficient cytoplasmic entry and stabilization of mRNA, we might expect cells infected with VA$^-$ mutants not to transport adenovirus mRNA rapidly because the viral mRNAs are not translated well in VA$^-$ cells. While the steady-state levels of late viral mRNAs are similar in wild-type virus- and VA$^-$ mutant infected cells, the initial rates of entry of viral mRNA in the VA$^-$ mutant-infected cells have not been reported.

An alternative possibility is that the viral RNAs that appear in the cytoplasm in the presence of the E1B-E4 protein complex have a better chance of becoming associated with the cytoskeletal framework of the host cell, which is where translation has been proposed to occur (7, 17). In preliminary studies comparing the cellular localization of viral and cellular mRNAs in uninfected, *dl*118, and *sub*309-infected HeLa cells, no accumulation of viral mRNAs in the soluble cell fraction was observed in *dl*118-infected cells,

which would be predicted if late mRNAs were not translated (data not shown).

In conclusion, it is pertinent to point out that the biggest difference in cells infected with wild-type and E1B mutant viruses detected in the present experiments is not in the transport of virus-specific RNAs, but in the steady-state levels of late viral mRNAs and the continued cytoplasmic accumulation of cellular mRNA species. This might imply that facilitation of the successful entry into the protein synthesis machinery accompanied by stabilization of the mRNA is the primary locus of E1B protein action. Furthermore, when sufficient successful association of viral mRNAs with the protein synthetic machinery has been established, inhibition of host protein results. Such an initial association of viral RNA with elements of cytoplasmic translation apparatus might not necessarily be automatically followed by translation. This would allow an E1B polypeptide in cells infected with VA^- mutants to stabilize viral mRNAs and perhaps integrate them partially into the translation apparatus, thus decreasing host protein translation. In fact, some viral protein synthesis does occur in VA^- mutant-infected cells. A necessary factor for rapid translation would then be supplied by VA and its associated proteins.

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