Characterization of the Translational Defect to Fiber Synthesis in Monkey Cells Abortively Infected with Human Adenovirus: Role of Ancillary Leaders

LAUREN SILVERMAN AND DANIEL F. KLESSIG*

The Waksman Institute, Rutgers, the State University of New Jersey, P.O. Box 759, Piscataway, New Jersey 08855-0759

Received 3 May 1989/Accepted 30 June 1989

Synthesis of the fiber protein of human adenovirus serotype 2 (Ad2) is 100-fold lower in abortively infected monkey cells, compared with productive infections, despite only a 5- to 10-fold reduction in fiber mRNA levels. Previously Anderson and Klessig (Proc. Natl. Acad. Sci. USA 81:4023-4027, 1984) demonstrated a direct correlation between the productive nature of the infection, efficient synthesis of the fiber protein *in vivo*, and the presence of the x or y ancillary leaders on 10 to 25% of fiber messages. To determine at what level in translation these leaders might be important, the relative rate of initiation and elongation of each class of fiber message was assessed. The presence of the y ancillary leader in productively infected cells increased the rate of initiation about twofold, although translational elongation was similar on all fiber messages. However, the rate of elongation of all fiber messages was threefold slower in abortively infected than in productively infected cells. This reduced elongation rate in abortive infections was specific for fiber. The similar distribution of fiber mRNAs on polysomes in both infections suggests that initiation must also be partially blocked in abortive infections. Since the majority of the fiber mRNA even in productive infections did not contain the ancillary leaders, the initiation and elongation defects in the abortive infection cannot be fully explained by the absence of these leaders. Therefore, other factors in the infected cell must be influencing the rate of translation.

Posttranscriptional regulation of protein synthesis can be exerted at several levels, including RNA processing (12, 16, 37, 47, 63), export from the nucleus (46, 53), messenger RNA stability (39, 49, 51), and translation (8, 30, 62). The extent of translational control of gene expression has only recently become apparent. Studies in the past few years have begun to define critical features of eucaryotic mRNAs that determine their differential translatability. This has led to the conclusion that the 5' ends of some mRNAs diminish their translation by the presence of secondary structure or multiple initiation codons (15, 35, 36, 56). Conversely, other messages contain specific sequences, structural features, or both at the 5' end which enhance initiation of translation. Examples of these positive elements include the Ω sequence of tobacco mosaic virus (60), the site of internal initiation of picornaviruses (27, 52), and the tripartite leader of adenovirus (42). Our present understanding of the role these *cis*-acting elements play, however, is far from complete.

We have been studying adenovirus replication in permissive and semipermissive host cells to gain additional insight into the mechanisms of translational regulation. Human adenovirus serotype 2 (Ad2) replicates efficiently in most cultured human cells (HeLa) but grows poorly in cell lines derived from African green monkey kidney cells (e.g. CV-1). Abortive infection of CV-1 cells by Ad2 results in an approximately 1,000-fold reduced yield of virus, compared with similar infections in human cells. However, Ad2 growth on monkey cells can be enhanced to normal levels by infection with host range (*hr*) mutants such as Ad2hr400, which contain a unique nucleotide change in the gene encoding the 72-kilodalton DNA-binding protein (5, 13, 31, 34).

Early events in the abortive viral life cycle proceed similarly to those in human cells infected with wild-type

virus or in monkey cells productively infected with *hr* mutants (3, 4, 21, 22, 65). The pattern of late viral protein synthesis, however, is significantly altered such that most late viral proteins are synthesized at levels which are 2- to 10-fold lower in abortive infections, compared with those in productive infections. This depression in protein synthesis parallels a comparable reduction in the steady state levels of the encoding late mRNAs (20, 32). The decreased levels of the late mRNAs are not caused by increased message turnover but by a 2- to 10-fold reduction in the rate of transcription of the major late viral transcription unit (28).

In contrast to the other late proteins, synthesis of the capsid protein fiber is reduced approximately 100-fold. The diminished level of fiber polypeptide cannot be completely explained by the 5- to 10-fold decrease in its mRNA level (5, 33). The discrepancy between fiber mRNA levels and fiber protein synthesis in abortively infected CV-1 cells could be caused by either depressed synthesis or reduced stability of the fiber protein. Several lines of evidence argue against the latter explanation, including pulse-chase analysis (3; L. Silverman, V. Cleghon, and D. F. Klessig, *Virology*, in press) and the readily detectable synthesis of fiber after microinjection of its mRNA (from productive or abortive infections) into abortively infected cells (7, 57).

In addition to the microinjection experiments, several other studies suggest that the monkey cell translational apparatus is competent to utilize fiber mRNA as a template. Nuclease treated, cell-free extracts prepared from mock- or from abortively infected CV-1 cells are able to translate exogenously added fiber mRNA isolated from Ad2-infected HeLa cells (55). Similarly, endogenous fiber mRNA is efficiently utilized during runoff translation in S10 extracts prepared from abortively infected CV-1 cells (5). Heterokaryons formed by fusing enucleated mock- or abortively infected monkey cells with nuclei from Ad2-infected human cells are also capable of synthesizing fiber (71).

* Corresponding author.

FIG. 1. The late transcription unit of Ad2. Fiber messages are represented as solid arrows; other late-message families are indicated by open arrows. All fiber messages contain the tripartite leader (1+2+3), while some may also contain the x, y, or z ancillary leaders. The synthetic oligonucleotide used for primer extension is represented by the open box labeled p. The top scale is in map units, while the bottom scale shows the number of base pairs (bp) from the *Eco*RI site at 75.9 map units (mu). The 19-kilodalton (19K) protein encoded by early region 3 (E3) is shown (▨).

Some of the above experiments that demonstrate functionality of ribosomes in abortively infected cells also indicate that fiber mRNA made in abortive infections is functional. This is further demonstrated by the fact that on a molar basis, it exhibits similar activity as that from RNA prepared from productive infections when added to a rabbit reticulocyte system in the form of purified message or cytoplasmic RNP complexes (5). Fiber mRNA isolated from both types of infections is capped and polyadenylated (33). However, there are some differences. In addition to the tripartite leader, common to all late viral messages, the fiber mRNA may also contain one of three ancillary leaders, x, y, and z. These leaders can be spliced between the tripartite leader and the coding exon for fiber (Fig. 1). Since these ancillary leaders are located 3' of the other late genes, they can be spliced only to the fiber mRNA. Fiber mRNA in productive infections differs from that found in abortive infections in respect to the presence of the x and y ancillary leaders. In productive infections in monkey or human cells, the x and y leaders are found on 10 to 25% of fiber messages. In contrast, little, if any, of these ancillary leaders are detected on fiber mRNA isolated from abortively infected cells (6; Silverman et al., in press). Therefore, x, y, or both may be necessary for efficient translation of fiber mRNA in vivo but not in vitro.

In this report, we have investigated the influence of the ancillary leaders on translation of the fiber message. Fiber mRNA in both productively and abortively infected cells was associated with polysomes, regardless of the presence of the ancillary leaders. While both initiation and elongation were defective in abortive infections, this could not be fully accounted for by the absence of x or y ancillary leaders. This suggests that other factors may be involved.

MATERIALS AND METHODS

Cells, viruses, and infections. Monolayer cultures of CV-1 cells were propagated in Dulbecco modified Eagle medium (Flow Laboratories, Inc., McLean, Va.) supplemented with 5% fetal calf serum (GIBCO Laboratories, Grand Island, N.Y.) plus 100 μ g of streptomycin per ml, 100 μ g of penicillin per ml, and 2 mM L-glutamine. Cells were infected as previously described (5) with 20 PFU per cell of Ad2 or Ad2hr400.

Polysome preparation. Cycloheximide was added to the medium of infected monolayers to a final concentration of 50 μ g/ml for 20 min, at 34 h postinfection. The cells (6×10^6) were washed twice, removed from tissue culture plates by scraping, and pelleted at $1,000 \times g$ for 5 min in phosphate-

buffered saline. This was followed by suspension in reticulocyte standard buffer (0.010 M Tris, pH 7.4, 0.010 M NaCl, 0.002 M $MgCl_2$). Heparan sulfate (0.5 mg/ml), Triton X-100 (0.5%), sodium deoxycholate (0.5%), and Tween 40 (1.0%) were added. The cells were then disrupted in a tight-fitting Dounce homogenizer, and the nuclei were pelleted at $750 \times g$ for 3 min. The supernatant was applied to a sucrose gradient (10 to 50% [wt/vol]) prepared in 0.010 M Tris (pH 7.6)-0.075 M KCl-0.003 M $MgCl_2$ and centrifuged at 36,000 rpm for 90 min at 4°C in a Beckman 41 rotor ($160,000 \times g$). Absorbance at 260 nm was monitored as gradients were fractionated by pumping from the bottom of the tube. For analysis of RNA species present in sucrose gradient fractions, sodium dodecyl sulfate was added to 0.5% and NaCl was added to 0.10 M, fractions were extracted twice with phenol-chloroform and twice with chloroform, and the nucleic acids were ethanol precipitated.

RNA analysis. Dot blot analysis was performed as described by Berry et al. (11). Hybridization was performed with a nick-translated plasmid containing Ad2 sequences specific for the main body of the fiber message (86.0 to 89.7 map units) (5). A 20-nucleotide primer with the sequence 5'-T-C-T-T-C-A-G-A-C-G-G-T-C-T-G-G-C-G-C-G-3' was synthesized by New England BioLabs, Inc., Beverly, Mass., and used for primer extension analysis. This primer is complementary to sequences beginning 7 nucleotides downstream from the splice junction at the 5' end of the main body of the fiber message. The single-stranded primer was labeled at the 5' end with T4 polynucleotide kinase and [γ - ^{32}P]ATP (43). Labeled primer (2 1/2 pmol) was combined with 20% of the RNA isolated per polysome gradient fraction in 0.1 ml of 0.10 M Tris (pH 8.3) and 0.0005 M EDTA, heated to 80°C for 5 min, and quick chilled on ice. The hybridization reaction buffer was brought to a final volume of 0.2 ml in a final concentration of 0.04 M KCl, 0.1 M Tris (pH 8.3), 0.01 M $MgCl_2$, 0.01 M dithiothreitol, 0.04 mg of dactinomycin per ml, and 0.001 M each of dATP, dCTP, dGTP, and dTTP. After hybridization for 2 h at 42°C in the presence of 5 U of avian myoblastosis virus reverse transcriptase (Life Sciences, Inc., St. Petersburg, Fla.), the reaction was terminated by the addition of 0.02 M EDTA, and RNA was hydrolyzed with 0.05 M NaOH and heating at 65°C for 15 min. Sodium dodecyl sulfate was added to the reaction mix to 0.5%, and the cDNA was phenol-chloroform extracted and ethanol precipitated. The labeled single-stranded products were electrophoresed in denaturing 8% polyacrylamide-urea gels (43) and detected by autoradiography.

Preparation of soluble and cytoskeletal fractions. Fractionation was performed essentially as describe by Lenk and Penman (38) and Ornelles et al. (48), with slight modifications of the lysis buffer. Cells were placed on ice and washed twice with phosphate-buffered saline. Monolayers were gently overlaid with 0.5 ml of lysis buffer (0.1 M KCl, 0.3 M sucrose, 0.01 M HEPES [*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; pH 7.4], 0.0025 M MgCl₂, 0.001 M EGTA [ethylene glycol-bis(β -aminoethyl ether)-*N,N,N'*, *N'*,-tetraacetic acid]) per 60-mm plate and incubated for 3 min. The soluble fraction (SOL) was removed directly from the culture plate. The material still adhering to the plate was gently washed again with lysis buffer and combined with the SOL fraction. The resulting cellular structures containing cytoskeletal frameworks (CSKs) and nuclei were suspended in 0.5 ml of detergent reticulocyte standard buffer (0.010 M NaCl, 0.010 M Tris [pH 7.4], 0.003 M MgCl₂, 0.5% sodium deoxycholate, and 1.0% Tween 40), with a rubber policeman used to dislodge them from the culture dishes. The cytoskeletal material was stripped from the nuclei by Dounce homogenization and pelleting at $1,000 \times g$ for 5 min. The CSK fraction was obtained as the supernatant. For RNA analysis, cell fractions were brought to 0.150 M NaCl, 0.100 M Tris, pH 7.6, and 1.0% sodium dodecyl sulfate. This was followed by phenol-chloroform extraction and ethanol precipitation.

Measurement of ribosome transit times. The procedure is based upon that of Fan and Penman (19) as modified by Palmiter (50). At 34 h postinfection, cells were labeled with [³H]leucine (10 μ Ci/ml for Ad2hr400-infected cells, 200 μ Ci/ml for Ad2-infected cells) in 1.0 ml of leucine-free media for 2 h at 37°C. The labeling media was removed, and cells were washed twice and maintained in medium containing 30 μ g of unlabeled leucine per ml for 1 h at 37°C. Cells were then labeled with [³⁵S]methionine (10 μ Ci/ml for Ad2hr400-infected cells, 200 μ Ci/ml for Ad2-infected cells) in 1.0 ml of methionine-free media. One 60-mm plate (10⁶ cells) was removed at 2-min intervals, treated with cycloheximide (50 μ g/ml), and placed on ice. All further manipulations were performed on ice or at 4°C. Cells were washed twice and scraped into phosphate-buffered saline. After pelleting, cells were suspended in 5 ml of isotonic buffer (0.150 M KCl, 0.010 M HEPES [pH 7.6], 0.0025 M MgCl₂, 0.150 M sucrose) plus 1.0% Nonidet P-40 and 1.0% sodium deoxycholate, held on ice for 5 min, and subjected to centrifugation at $1,000 \times g$ for 5 min at 4°C. Supernatants were removed and aliquots were precipitated with 10% trichloroacetic acid to determine total incorporation of [³H]leucine and [³⁵S]methionine. A second sample was subjected to centrifugation at 45,000 rpm for 2 h at 4°C in a Beckman SW 50.1 rotor. Incorporation into nonpolysome-associated polypeptides was measured by trichloroacetic acid precipitation of the resulting postpolysomal supernatant. To measure the average ribosome transit time of a particular protein, the supernatant centrifuged at 45,000 rpm was immunoprecipitated and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis to exclude cross-contaminating material. Size markers were used to determine the location of the radiolabeled proteins of interest. Radioactivity was eluted from the gel by using NCS solubilizer (Amersham Corp., Arlington Heights, Ill.) and the OCS (Amersham) scintillation system.

RESULTS

Synthesis of fiber protein and association of fiber mRNA with polysomes. Abortively infected monkey cells synthesize

FIG. 2. Association of fiber mRNA with polysomes and release by puromycin in productively and abortively infected CV-1 cells. Ad2- or Ad2hr400-infected CV-1 cells were treated without (upper panels) or with (200 μ g/ml, 45 min; lower panels) puromycin prior to preparation of cell extracts. Cytoplasmic extracts were then subjected to centrifugation on 10 to 50% sucrose gradients at $160,000 \times g$ for 90 min. The resulting sedimentation profiles are shown. The gradients were divided into 12 fractions of equal volume. RNA was isolated from each fraction, and equal portions (20%) of each fraction were analyzed for fiber mRNA with a nick-translated probe specific to the main body of the fiber message (86.0 to 89.7 map units). The distribution of fiber message, as determined by dot blot, is located under the corresponding gradient profile. Note that the Ad2 dot blots were exposed for a longer period of time to obtain dot intensities similar to those obtained with Ad2hr400. RNPs, Ribonucleoproteins. A₂₆₀, Absorbance at 260 nm.

much lower levels of fiber protein than do productively infected cells. To assess the extent of this difference, synthesis of the fiber protein was analyzed during Ad2 and Ad2hr400 infections and was found to be depressed approximately 100-fold in Ad2-infected cells, compared with those infected with Ad2hr400 (data not shown). However, the level of fiber message in Ad2-infected cells was only 10-fold lower than that in Ad2hr400-infected cells (data not shown), as reported previously (5). Therefore, the 100-fold-lower synthesis of fiber in abortively infected cells was only partly accounted for by reduced levels of the encoding mRNA.

To determine whether fiber mRNA from abortively infected as well as productively infected cells was associated with ribosomes, detergent-solubilized cytoplasmic fractions were prepared from Ad2- and Ad2hr400-infected CV-1 cells at 34 h postinfection and subjected to sedimentation through 10 to 50% sucrose gradients. The gradients were fractionated, and the RNA from each fraction was subjected to dot blot analysis (Fig. 2, top panel). The distribution of fiber mRNA among heavy and light polysomes, monosomes, and ribonucleoproteins was similar in productively and abortively infected cells (Table 1). As an internal control, the hexon mRNA, which is not subject to translational control, was analyzed. It exhibited a distribution similar to fiber messages (data not shown).

TABLE 1. Distribution of fiber mRNA in sucrose gradients in the presence or absence of puromycin^a

Virus	Presence (+) or absence (-) of puromycin	% Fiber mRNA in fractions:					
		12 and 11	10 and 9	8 and 7	6 and 5	4 and 3	2 and 1
Ad2hr400	-	11	11	26	18	18	16
Ad2hr400	+	10	19	36	23	6	6
Ad2	-	7	7	22	28	23	13
Ad2	+	6	16	34	32	7	5

^a Infected cells were treated with or without puromycin, and extracts were prepared and applied to 10 to 50% sucrose gradients as described in Fig. 2. Gradients were separated into fractions of equal volume, and RNA was isolated. The fiber mRNA content was determined by dot blot analysis. The radioactivity in each fraction of the gradient was determined by cutting out the individual dots and counting in Aquasol-2 (Dupont, NEN Research Products, Boston, Mass.). The percent total radioactivity in each portion of the gradient was tabulated.

A large proportion of the fiber mRNA in both infections was located in the 80S region of the gradient. To obtain better resolution of this region, infected cell extracts were compared on sucrose gradients with extended centrifugation time to expand the 80S region. Once again, the distribution of fiber mRNA was found to be similar in Ad2- and Ad2hr400-infected cells (data not shown).

To show that mRNA is associated with authentic polysomes, puromycin release experiments were done. Puromycin causes release of nascent polypeptide chains and the disruption of polysomes. The distribution of fiber mRNA was again analyzed on sucrose gradients with or without puromycin treatment prior to preparation of the cytoplasmic extract (Fig. 2, bottom panel). Fiber mRNA from both infections was released from polysomes to similar extents (Table 1). Since sensitivity to puromycin requires the formation of at least a single peptide bond, fiber mRNA in abortively infected cells was associated with polysomes that had the capacity to participate in at least limited elongation. The similar distribution in the two types of infections of fiber mRNA on ribosomes that exhibit similar sensitivity to puromycin, together with our previous translational runoff experiments (5), indicate that the large reduction in fiber synthesis in abortive infections is not caused by lack of association of this mRNA with ribosomes.

Distribution of fiber mRNA between the soluble and cytoskeletal fractions. The work of Penman and colleagues (14, 38, 48) suggests that actively translating polysomes are preferentially bound to the CSK. This conclusion was based upon studies of virus-infected cells (poliovirus, vesicular stomatitis virus) which exhibited a temporal correlation between synthesis of viral proteins (or depression of host protein production) and association of their mRNAs with the CSK. Additional evidence suggests that ribosomes are bound to the CSK via the mRNA which they are translating (14).

In productive infections of monkey or human cells, where the fiber protein is efficiently synthesized, a substantial portion (10 to 25%) of fiber messages contain either the x or y ancillary leaders. In contrast, during abortive infections very little of the mRNA carries either of these ancillary leaders, suggesting that these sequences may be necessary for efficient translation of the fiber mRNA. If x, y, or both leaders enhance translational efficiency by preferential interaction with the CSK, then in the productively infected cells the majority of fiber messages containing these leaders should be CSK associated. To test this hypothesis, the leader composition of the fiber mRNA from both the SOL

FIG. 3. Primer extension analysis of the 5' ends of fiber messages isolated from the SOL and CSK fractions of productively infected cells. SOL and CSK fractions were prepared from Ad2hr400-infected cells as described in Materials and Methods. The isolated RNA was analyzed by primer extension. The synthetic oligonucleotide used for primer extension is represented by the open box labeled p in Fig. 1. Reaction products were fractionated on an 8% polyacrylamide-urea gel by electrophoresis and then subjected to autoradiography. Bands corresponding to fiber messages containing different 5' leader segments are labeled at the right.

and CSK fractions of productively infected cells was determined by primer extension (Fig. 3). The primer was complementary to sequences adjacent to the 5' splice junction of the main body of the fiber message, as illustrated in Fig. 1. The sizes of the major leader segments have been determined previously (1, 6, 23, 24, 64, 70). Therefore, the presence of each of these leaders was readily identified as cDNA products with appropriate mobilities in denaturing urea-acrylamide gels.

Approximately 40% of the fiber mRNA was found associated with the SOL fraction, and 60% was found associated with the CSK fraction in Ad2hr400-infected cells (Fig. 3). A similar distribution was observed in abortive infections by Ad2 (data not shown). Furthermore, fiber mRNA isolated from the SOL and CSK fractions of productively infected cells contained similar proportions of each 5' end. Therefore, the more efficient synthesis of fiber protein in Ad2hr400-infected cells could not be explained by enhanced interaction of the y, x, or both leaders with the CSK of the cell.

In addition to the tripartite leader and leaders that consist of the tripartite leader plus x, y, or z, several larger, less prominent bands were also seen (Fig. 3). These are probably the cDNA products of fiber mRNA with the tripartite leader plus a combination of the ancillary leaders or the tripartite leader plus one of the ancillary leaders and the i leader which is encoded between leaders 2 and 3 of the tripartite leader (Fig. 1). The infected monkey cells also synthesized small amounts of fiber mRNA whose corresponding cDNAs migrated just above the tripartite leader band or between those containing the y and z leaders, respectively. Their origin is unknown, although they probably represent 5' ends of fiber mRNA because no background bands were seen when RNA from uninfected cells was used in the primer extension reaction. They were present in similar amounts in productive

FIG. 4. Primer extension analysis of the 5' ends of fiber messages associated with polysomes in Ad2hr400- and Ad2-infected cells. Polysome profiles were prepared, and RNAs were isolated as described in Fig. 2. Equal proportions (20%) of the RNA from each gradient fraction was analyzed by primer extension. The cDNA products corresponding to fiber messages with different 5' leaders were as indicated. Note that the Ad2 gel was exposed for a longer period of time to obtain band intensities similar to those obtained with Ad2hr400.

and abortive infections (Fig. 4) and were not further characterized.

Analysis of the leaders on fiber messages associated with polysomes. Messages engaged in high levels of translational activity may be distinguished from less active messages by their association with polysomes of different sizes. The size of the polysomes, assuming the same length of mRNA, reflects a balance between the rate of initiation and elongation. A message with a relatively high rate of initiation and moderate or slow rate of elongation would be overrepresented in the heavier portion of a polysome profile. Similarly, a message that initiates poorly or initiates with moderate kinetics but elongates with rapid kinetics should be associated with smaller polysomes. To gain insight into the behavior of fiber mRNA with alternative leaders, the RNA isolated from sucrose gradients was analyzed by primer extension (Fig. 4; Table 2). The fiber species containing only the tripartite leader was distributed in a similar manner in both productively and abortively infected cells. This distribution was expected given that (i) the dot blot analysis already showed a similar distribution for total fiber mRNA in the two types of infections and (ii) this mRNA class represents the majority of the fiber messages in both types of infections. In productively infected cells, there was a two-fold enrichment of y-containing fiber mRNA on heavier polysomes while fiber mRNA containing the z leader was underrepresented in this region. Messages with the x leader appeared to be equally distributed throughout the polysome profile; however, their low levels made quantitation difficult and therefore less reliable. Unlike the productively infected cells, in abortively infected cells z-containing messages were well represented on heavy polysomes. Thus, fiber transcripts with the z leader were distributed differently within

TABLE 2. Distribution of Ad2hr400 fiber mRNAs with different 5' ends in sucrose gradients^a

Fraction	% Fiber mRNA with 5' end ^b :			
	123	123X	123Z	123Y
1	48	4	7	41
2	54	3	7	36
3	61	3	5	31
4	63	3	5	29
5	59	5	15	21
6	45	8	25	22
7	47	7	26	20
8	48	9	23	20
9	54	6	23	17
10	58	6	21	15
11	57	6	20	17
12	56	7	20	17

^a Autoradiograms of the primer extension products were analyzed by densitometry of films exposed in the linear range.

^b The percentage of fiber mRNA with different 5' ends was determined for the major bands corresponding to 123, 123X, 123Z, and 123Y only. The percent contribution of other bands in the gel were not included in this analysis. Note that the heaviest polysomes (corresponding to fractions 1 to 3) contain on a percent basis about twice as much y-containing fiber message as do other fractions in the gradient.

alternative infectious environments, and messages with different leaders in the same productive host also showed different distributions.

Relative rate of initiation on fiber mRNA with different leaders. To determine the relative efficiency of initiation of fiber messages with different leaders, we used the cycloheximide procedure of Fan and Penman (19). At low concentrations, this drug slows the rate of elongation without affecting the rate of initiation. This can lead to an increase in the number of ribosomes associated with message. A message which is maximally initiated cannot be loaded with more ribosomes during this treatment, while a less efficiently initiated message will have ribosomes added to it.

Cytoplasmic extracts were prepared from productively infected cells, treated with different concentrations of cyclo-

FIG. 5. Determination of the relative rate of initiation on fiber mRNA with different leaders. At 34 h postinfection, cells infected with Ad2hr400 were treated with cycloheximide (1.00 or 0.25 μ g/ml for 20 min) or were left untreated (0.00 μ g/ml). At the end of this period, cycloheximide was added to a final concentration of 50 μ g/ml. Infected cell extracts were prepared and applied to 10 to 50% sucrose gradients. Each gradient was divided into 12 fractions of equal volume and analyzed for the presence of the different 5' ends of fiber mRNA by primer extension analysis. The results of analysis of the polysome region are shown, in which lanes denoted by 1 contain the heaviest polysomes and those denoted by 6 contain the lightest polysomes. Bands corresponding to fiber messages containing different 5' leader segments are labeled at the right.

TABLE 3. Analysis of ratios of different 5' ends after treatment with cycloheximide and ribosome recruitment onto messages with different 5' ends^a

5' End	Ratio of band intensity for indicated fractions after treatment with cycloheximide at:								Relative ribosome addition ^b
	1.00 μg/ml				0.25 μg/ml				
	4	3	2	1	4	3	2	1	
123Y	1.5	4.1	1.2	1.2	1.4	2.4	1.2	1.1	0.5
123Z	1.8	1.9	1.4	1.1	1.7	1.4	1.2	1.5	0.4
123X	1.2	1.3	1.0	0.9	1.3	1.4	1.1	1.3	0.3
123	1.8	5.7	4.9	3.4	1.6	4.5	4.1	2.8	1.0

^a Autoradiograms of primer extension products in Fig. 5 (lanes 1 to 4 of each gradient) were analyzed by densitometry.

^b The addition of ribosomes was determined by measuring the ratio of individual 5' ends in equivalent fractions in the presence and absence of cycloheximide. The relative ribosome addition was determined by comparing the sum of the ratio of the band intensities for a given 5' end in fractions 1 to 4, relative to the sum of band intensities for message with the 5' end 123, after treatment with cycloheximide.

heximide, and fractionated on sucrose gradients. The extent of ribosome addition onto each class of fiber message was determined by comparing the amounts associated with heavy polysomes (Fig. 5, fractions 1 to 4; Table 3) in the presence of cycloheximide with that in the absence of cycloheximide. Cycloheximide treatment produced a significant increase in ribosome addition to messages with the tripartite leader. This was two- to threefold greater than the extent of ribosome addition to messages with the ancillary leaders. Therefore, fiber messages with the leaders x, y, or z appeared to initiate with greater efficiency than did the majority of fiber messages. This may explain the twofold overrepresentation of y messages in the heavier portion of the polysome profile, as described in the previous section. However, this is inconsistent with the poor representation of z-containing mRNA on large polysomes. We do not have an explanation for this inconsistency.

Relative elongation rates on fiber mRNA with different leaders. In a complementary set of experiments, the relative rates of elongation on fiber mRNA with different leaders was determined under conditions in which initiation but not elongation was inhibited. When initiation is inhibited with low levels of pactamycin, messages become associated with smaller and smaller polysomes and eventually are completely dissociated from ribosomes. The rate of this progression is faster on messages that are more rapidly traversed by ribosomes. Thus the effect of 5' leaders on rates of elongation could be determined by comparing their distribution among various sizes of polysomes, monosomes, and free ribonucleoproteins after limited time periods of pactamycin treatment. Productively infected CV-1 cells were treated with pactamycin and allowed to continue runoff translation for 0 to 60 s. Densitometry analysis of the primer extension products made from mRNA associated with various sizes of polysomes (Fig. 6) indicated that in productively infected cells, messages with different leaders exhibited similar rates of transition from large to small polysomes in the presence of pactamycin. Therefore, the 5' leaders of fiber mRNA do not affect the rate of translational elongation. These results are also consistent with observation that y-containing mRNAs are both more efficiently initiated and overrepresented on large polysomes.

Relative elongation rates in productive and abortive infections. The observation that fiber mRNA was on polysomes that had already initiated translation in abortively infected

FIG. 6. Determination of the relative rate of elongation on fiber mRNA with different leaders. At 34 h postinfection, Ad2hr400-infected CV-1 cells were treated with 0.1 μ M pactamycin for 0, 40, or 60 s, as indicated. At the end of the limited runoff period, pactamycin was added to a final concentration of 100 μ M and cycloheximide was added to 50 μ g/ml. Cell extracts were prepared and applied to 10 to 50% sucrose gradients. Each gradient was divided into 12 fractions of equal volume and analyzed for the presence of the different 5' ends of fiber by primer extension. The results of analysis of the polysome region are shown, in which lanes denoted by 1 contain the heaviest polysomes and lanes denoted by 6 contain the lightest polysomes. Bands corresponding to fiber messages containing different 5' leader segments are labeled at the right.

cells suggested that part of the block to fiber synthesis in these cells must be at the level of elongation. The relative rate of elongation of the fiber polypeptide in Ad2- and Ad2hr400-infected cells was thus investigated. This was determined by comparing the average ribosome half-transit time to the half-transit time of ribosomes specifically synthesizing fiber in productive and abortive infections. By using the procedure of Fan and Penman (19) as modified by Palmiter (50), the rate of completion of nascent polypeptides (from a polysome-bound to a polysome-free state) was measured. At 34 h postinfection, cells were labeled with [³H]leucine for 2 h. After a 1-h chase, [³⁵S]methionine was added and samples were removed at 2-min intervals. Each sample was analyzed for the amount of radioactivity incorporated into total polypeptides (nascent as well as completed) and that incorporated into completed, free protein that remained in the postribosomal supernatant. Labeling of specific proteins such as fiber was determined by immunoprecipitation of the completed polypeptides from the postribosomal supernatant (details given in Materials and Methods). When the results were plotted as a function of time, parallel lines were generated (Fig. 7). The distance between total and free polypeptides is one-half the average ribosome transit time. Similarly, the distance between total and a specific polypeptide is one-half the ribosome transit time for synthesis of that particular protein. Virtually identical ribosome transit times were obtained for overall protein synthesis in productively and abortively infected cells. Likewise, the transit times for ribosomes translating hexon and DNA-binding protein were similar in both types of infections. Synthesis of fiber, however, was approximately threefold slower in Ad2-infected cells than in Ad2hr400-infected cells.

To further demonstrate the translational elongation defect in fiber synthesis in abortive infections, we compared the relative elongation rates on total fiber mRNA in abortive infections versus those in productive infections under conditions in which initiation but not elongation was inhibited with pactamycin (as described in the previous section). Fiber mRNA was released from heavy polysomes with kinetics at least threefold slower in Ad2-infected cells than in

FIG. 7. Average ribosome transit times in Ad2hr400- and Ad2-infected cells. At 34 h postinfection, cells were labeled with [^3H]leucine for 2 h. After a 1-h chase period, [^{35}S]methionine was added and samples were removed at the times indicated. The ^{35}S labeling: ^3H labeling ($^{35}\text{S}/^{3\text{H}}$) ratios for total labeled ($\blacklozenge$) and soluble (∇ , non-polysome-associated) polypeptides are plotted as a function of time. The distance between these two parallel lines represents one-half the average ribosome transit time. The soluble fraction was also analyzed for newly synthesized fiber ($\blacktriangle$), hexon ($\blacksquare$), and DNA-binding protein ($\circ$). The distance between these parallel lines and the line generated for total polypeptide synthesis represents one-half the average ribosome transit time for ribosomes engaged in the translation of each of the corresponding messages.

Ad2hr400-infected cells (Fig. 8). As a control, the relative elongation rate on hexon messages was also determined and found to be the same in both productively and abortively infected cells. These results corroborate those obtained by the measurement of ribosome half-transit times.

Thus, an elongation defect contributes to the block to fiber synthesis in abortively infected cells. However, since the distribution of fiber mRNA among large and small polysomes and monosomes was similar in productive and abortive infections, there must also be a compensatory initiation defect in abortively infected cells. Together, these two defects contribute to the low level of fiber synthesis in abortively infected cells.

DISCUSSION

The experiments presented above address the disparity between the rate of fiber protein synthesis and the amount of

fiber mRNA in abortively infected monkey cells. Fiber protein synthesis is 100-fold lower in Ad2-infected cells than in cells productively infected with Ad2hr400. This apparent difference in synthesis cannot be accounted for by the 5- to 10-fold reduction in the steady state level of the encoding mRNA. Moreover, different stabilities of newly synthesized fiber have been excluded.

The fiber mRNA in abortive infections was associated with polysomes. The distributions of this mRNA in the polysomes, monosomes, and free ribonucleoprotein region of the sucrose gradients were similar for abortive and productive infections. The message was on authentic ribosomes as defined by their sensitivity to puromycin. In addition, the large amount of fiber mRNA migrating in the 80S region of the gradient appeared to be associated with ribosomes, since pronase treatment at 4°C (which disrupts ribonucleoprotein aggregates but not monosomes [25, 59, 68]) did not alter the distribution of the fiber mRNA (data not shown). Moreover, Anderson and Klessig (5) demonstrated that these ribosomes had initiated translation *in vivo* and were active in translational runoff *in vitro*. Penman and colleagues (14, 38, 48) have suggested that actively translating polysomes are preferentially associated with the CSK. However, we found that in both types of infections the fiber mRNA was similarly distributed between the soluble and cytoskeletal fractions of the cell.

In productive infections, a significant portion (10 to 25%) of fiber messages contain the x and y ancillary leaders, while very few fiber messages in abortively infected cells harbor these sequences (6; Silverman et al., *in press*). The correlation between efficient synthesis of fiber protein and the presence of x and y prompted us to investigate their possible roles in translation. Fiber mRNAs with only the tripartite leader or with this leader plus either of the three ancillary leaders, x, y, or z showed the same distribution between the cytoskeletal and soluble fractions of productively infected monkey cells. In contrast, while all four classes of messages were associated with polysomes, the mRNA containing the y leader was somewhat overrepresented on larger polysomes. This was consistent with the observations that (i) translational initiation was about twofold more rapid on it than on fiber mRNA with only the tripartite leader and (ii) elongation

FIG. 8. Relative rate of elongation of fiber and hexon mRNA in Ad2hr400- and Ad2-infected cells. Limited elongation was performed as described in Fig. 9; however, the periods of elongation were 0 and 20 s for Ad2hr400 and 0 and 60 s for Ad2. After gradient centrifugation, the bottom six fractions in each polysome profile were analyzed for fiber and hexon mRNA (with 20% of the RNA in each fraction) by dot blot analysis. The fiber probe was the same as that used in Fig. 2; the hexon probe extends from 51.0 to 59.5 map units. Note that the Ad2 dot blots were exposed for a longer period of time to obtain intensities of exposure similar to those obtained with Ad2hr400.

rates were similar on all four classes of mRNA in productive infections. The rate of elongation in abortive infections, however, was at least threefold slower than in productive infections, and this reduction was specific for the fiber polypeptide. This slower rate of elongation combined with the 10-fold-lower level of fiber mRNA may account for much of the 100-fold reduction in fiber synthesis.

While fiber messages containing the tripartite leader and the ancillary leaders x, y, and z have been the primary focus of this investigation, the possibility remains that fiber mRNAs with unidentified leaders might also direct the synthesis of fiber in monkey cells. However, most of the fiber mRNAs with unidentified leaders are present in similar proportions in productive and abortive infections and, therefore, probably do not account for the different levels of fiber in these cells. One fiber message that differs in concentration in these infections may correspond to a combination of the x and y leaders (as determined by size) which would be expected to be found in lower amounts in abortively infected cells. That the mRNAs with the leaders we have analyzed in detail direct the synthesis of fiber, under at least some conditions, has been demonstrated by Dunn et al. (18), who showed that messages with the tripartite leader or the tripartite leader plus y can be translated *in vitro*. Microinjection data (7) also demonstrated that a message population containing the tripartite leader and the tripartite leader plus z could be translated in monkey cells. Furthermore, ongoing experiments with fiber cDNA copies indicate that genes encoding fiber messages with the tripartite leader alone or with the additional leaders x and y give similar protein levels in a transient expression assay (D. E. Brough and D. F. Klessig, unpublished results).

Our analysis indicates that despite the previous correlation between the presence of the x and y ancillary leaders and efficient synthesis of fiber, the difference in the leader structure of fiber mRNA in the two types of infections cannot alone account for poor utilization of this mRNA in abortive infections. First, the majority of the fiber mRNA in abortive (~80%) and productive (~60%) infections contained only the tripartite leader and was on polysomes. The y and x classes which were essentially absent in abortive infections represented approximately 20 and 5%, respectively, of the fiber mRNA in productive infections and initiated translation no more than threefold better than did the class with the tripartite leader alone. Thus, their low levels and only modest increase in initiation activity could not account for the much higher rate of fiber synthesis in productive infections. Second, while the rate of elongation of fiber (but not other proteins) was severalfold slower in abortive infections, this appeared to be independent of leader structure. In productive infections, the elongation rate was the same for all four classes of mRNA. Furthermore, the majority (~60 to 80%) of the fiber mRNA in both infections had the same leader structure, yet the average elongation rate was significantly different.

The tripartite leader enhances translation of some (10, 42) but not all mRNAs (2, 44) at late times after adenovirus infection. Thus, the effect of the tripartite leader is probably dependent upon additional features of the adjoining RNA. It has been suggested that an mRNA which is a potent initiator competes effectively with messages that contain the tripartite leader, whereas if it is a poor initiator, then addition of the tripartite leader to it improves translation (2). The tripartite leader can also facilitate cap-independent translation (17). This could ensure efficient translation by circumventing a limited availability of certain initiation factors

(cap-binding protein complex). While previous studies have indicated that fiber mRNA with only the tripartite leader is readily translated *in vitro* (5, 18), here we demonstrate that it is associated with active polysomes and presumably also translated *in vivo*. This argues that the 203-nucleotide tripartite leader is sufficient for translation of the fiber mRNA. What role then do the ancillary leaders play? Addition of at least the 184 nucleotide y leader appears to enhance initiation about twofold. However, its effect on total fiber synthesis is probably rather modest since this class represents only 20% of fiber mRNA. Thus, it is unlikely that the altered leader splicing pattern is the primary factor responsible for the poor translation of the fiber message in abortive infections. Rather, our data suggest that the poor translation of the fiber message may be caused by differences in the intracellular environments of the two types of infections. For instance, we have noted that fiber messages with the z ancillary leader are distributed differently in productive and abortive infections, being less prevalent on larger polysomes in productively infected cells. Since z contains an open reading frame 5' and slightly overlapping with the open reading frame encoding fiber, perhaps ribosomes in these two types of infected cells handle this polycistronic message in a slightly different manner.

If the infectious environment plays a key role in translational regulation, why is only fiber affected? Selective translation of cellular as well as viral messages has been reported in several systems. Translational control at the level of initiation has been described for reovirus in infected mouse cells (9), ribosomal proteins in glucocorticoid-treated P1798 cells (45), the herpes simplex polymerase gene (69), and actin mRNA under conditions of heat shock (62). Modulation at the level of elongation has been reported for hsp 70 (61) and tyrosine aminotransferase (58). Translational control at the level of both initiation and elongation has been described for nonheat shock messages at high temperature (8), for light-regulated expression of ribulose-1,5-bisphosphate carboxylase in amaranth (11), and for cellular messages after infection by influenza virus or adenovirus (29).

The unusual nature of fiber may provide clues to the mechanism(s) governing its synthesis. Fiber is the only adenovirus protein known to undergo O-linked glycosylation (26). Perhaps O glycosylation and translation are linked. If this type of glycosylation were perturbed in abortive infections, it might impair translational elongation of the fiber polypeptide. For instance, maybe fiber elongation is limited by an unfavorable conformation of the growing peptide chain, which can be relieved by carbohydrate addition. It has been suggested that translational pausing along the message may facilitate folding of polypeptides. Such discontinuous translation is not incompatible with efficient translation (40, 54, 67). Therefore, modification of the protein could potentially promote the correct tertiary conformation for continued translation of fiber.

In addition to the slower fiber elongation rate in abortive infections, a compensatory retardation in initiation also must be occurring because the distribution of fiber mRNA on different sizes of polysomes was similar in abortive and productive infections. Several possibilities could account for this specific reduction of fiber initiation. First, the observation that the bulk of adenovirus messages are on smaller polysomes than expected during the late phase in productive (29, 66; our data) and abortive (our data) infections suggests that initiation on the viral mRNAs is inefficient, elongation is unusually rapid, or both. Since our ribosome transit time measurements are in good agreement with those determined

for other systems (19, 50, 59), inefficient initiation is most likely. In abortively infected cells, a slight decrease in general initiation could be magnified for fiber. Changes in the level of a rate-limiting factor preferentially affects translation of mRNAs for which the factor has a lower affinity (41). If the putative factor is more limiting in abortive than in productive infections and if it has a lower affinity for fiber mRNA than for the large pool of other mRNAs, then depression of fiber synthesis in abortive infections will be much more pronounced than for other proteins. Alternatively, although the factor may be present in adequate quantities in the cell, it may not be fully accessible to the fiber mRNA and its associated ribosomes because of their subcellular locations. The ability to overcome the translation block, either by microinjection of the fiber mRNA into the cytoplasm of abortive infections (7) or by disruption of the cells during runoff translation in cell-free extracts (5), is consistent with possible differences in subcellular location in the two types of infections. This might be related to the O-linked glycosylation of fiber, if this modification occurs in a special compartment of the cell. A third possibility is the presence of a negative effector in abortive infections to which fiber synthesis is particularly sensitive. However, results of two experiments argue against, but do not strictly preclude, this model. Fiber synthesis could be readily detected after microinjection of the message into abortively infected cells (7, 57) or in heterokaryons resulting from fusion of infected HeLa nuclei with enucleated monkey cells (71).

Whatever the factor(s) involved, the exquisite sensitivity of the fiber message should provide a useful tool in determining additional sequence structural features of an mRNA which are subject to translational regulation. Characterization of these regions may allow the identification of cellular factors responsible for this level of control.

ACKNOWLEDGMENTS

We thank D. Breiding and J. Berry for helpful discussion and advice.

This work was supported by Public Health Service grant AI23591 from the National Institutes of Health and grant MV-407 from the American Cancer Society to D.F.K. D.F.K. is a recipient of Faculty Research Award 270 from the American Cancer Society.

LITERATURE CITED

1. Akusjarvi, G., and U. Pettersson. 1979. Sequence analysis of adenovirus DNA: complete nucleotide sequence of the spliced 5' noncoding region of adenovirus 2 hexon messenger RNA. *Cell* 16:841-850.
2. Alonso-Caplen, F. V., M. G. Katze, and R. M. Krug. 1988. Efficient transcription, not translation, is dependent on adenovirus tripartite leader sequences at late times of infection. *J. Virol.* 62:1606-1616.
3. Anderson, C. W., M. M. Hardy, and J. B. Lewis. 1988. Abnormal expression of a late gene family L1 protein in monkey cells abortively infected with adenovirus type 2. *Virus Genes* 1:149-164.
4. 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.
5. Anderson, K. P., and D. F. Klessig. 1983. Posttranscriptional block to synthesis of a human adenovirus capsid protein in abortively infected monkey cells. *J. Mol. Appl. Genet.* 2:31-43.
6. Anderson, K. P., and D. F. Klessig. 1984. Altered mRNA splicing in monkey cells abortively infected with human adenovirus may be responsible for inefficient synthesis of the virion fiber polypeptide. *Proc. Natl. Acad. Sci. USA* 81:4023-4027.
7. Anderson, K. P., E. A. Wong, and D. F. Klessig. 1985. Microinjection of mRNA enhances the translational efficiency of human adenovirus fiber message in monkey cells. *Mol. Cell. Biol.* 5:2870-2873.
8. Ballinger, D. G., and M. L. Pardue. 1983. The control of protein synthesis during heat shock in *Drosophila* cells involves altered polypeptide elongation rates. *Cell* 33:103-114.
9. Benavente, J., and A. J. Shatkin. 1988. Avian reovirus mRNAs are nonfunctional in infected mouse cells: translational basis for virus host-range restriction. *Proc. Natl. Acad. Sci. USA* 85:4275-4261.
10. Berkner, K. L., and P. A. Sharp. 1985. Effect of the tripartite leader on synthesis of a non-viral protein in an adenovirus 5 recombinant. *Nucleic Acids Res.* 13:841-857.
11. Berry, J. O., J. P. Carr, and D. F. Klessig. 1988. mRNAs encoding ribulose-1,5-bisphosphate carboxylase remain bound to polysomes but are not translated in amaranth seedlings transferred to darkness. *Proc. Natl. Acad. Sci. USA* 85:4190-4194.
12. Breibart, R. E., A. Andreadis, and B. Nadal-Ginard. 1987. Alternative splicing: a ubiquitous mechanism for the generation of multiple protein isoforms from a single gene. *Annu. Rev. Biochem.* 56:467-495.
13. Brough, D. E., S. A. Rice, S. Sell, and D. F. Klessig. 1985. Restricted changes in the adenovirus DNA binding protein leading to extended host range or temperature-sensitive phenotypes. *J. Virol.* 55:206-212.
14. 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* 23:113-120.
15. Chevrier, D., C. Vezina, J. Bastille, C. Linard, N. Sonenberg, and G. Boileau. 1988. Higher order structures of the 5'-proximal region decrease the efficiency of translation of the porcine proopiomelanocortin mRNA. *J. Biol. Chem.* 263:902-910.
16. Danner, D., and P. Leder. 1985. Role of an RNA cleavage/polyadenylation site in the production of membrane bound and secreted IgM mRNA. *Proc. Natl. Acad. Sci. USA* 82:8658-8662.
17. Dolph, P. J., V. Racaniello, A. Villamarin, F. Palladino, and R. J. Schneider. 1988. The adenovirus tripartite leader may eliminate the requirement for cap-binding protein complex during translation initiation. *J. Virol.* 62:2059-2066.
18. Dunn, A. R., M. B. Mathews, L. T. Chow, J. Sambrook, and W. Keller. 1978. A supplementary adenoviral leader sequence and its role in messenger translation. *Cell* 15:511-526.
19. Fan, H., and S. Penman. 1970. Regulation of protein synthesis in mammalian cells. II. Inhibition of protein synthesis at the level of initiation during mitosis. *J. Mol. Biol.* 50:655-670.
20. Farber, M. S., and S. G. Baum. 1978. Transcription of adenovirus RNA in permissive and nonpermissive infection. *J. Virol.* 27:136-148.
21. Feldman, L. A., J. S. Butel, and F. Rapp. 1966. Interaction of simian papovavirus and adenovirus. Induction of adenovirus tumor antigen during abortive infection of simian cell. *J. Bacteriol.* 91:813-818.
22. 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 cells. *J. Virol.* 5:586-597.
23. Herisse, J., G. Courtois, and F. Galibert. 1980. Nucleotide sequence of the EcoRI D fragment of adenovirus 2 genome. *Nucleic Acids Res.* 8:2173-2192.
24. Herisse, J., and F. Galibert. 1981. Nucleotide sequence of the EcoRI E fragment of adenovirus 2 genome. *Nucleic Acids Res.* 9:1229-1240.
25. Herzberg, M., and H. Breibart. 1980. Block in the elongation of protein synthesis in rabbit reticulocytes by the action of the ionophore valinomycin. *Mol. Biol. Rep.* 6:163-167.
26. Ishibashi, M., and J. V. Maizel. 1974. The polypeptides of adenovirus. VI. Early and late glycoproteins. *Virology* 58:345-361.
27. Jang, S. K., H. G. Kräusslich, M. J. H. Nicklin, G. M. Duke, A. C. Palmenberg, and E. Wimmer. 1988. A segment of the 5' nontranslated region of encephalomyocarditis virus RNA directs internal entry of ribosome during in vitro translation. *J. Virol.* 62:2636-2643.

28. Johnston, J., K. P. Anderson, and D. F. Klessig. 1985. Partial block to transcription of human adenovirus late genes in abortively infected monkey cells. *J. Virol.* **58**:378–385.
29. Katze, M. G., D. Decorato, and R. M. Krug. 1986. Cellular mRNA translation is blocked at both initiation and elongation after infection by influenza virus or adenovirus. *J. Virol.* **60**:1027–1039.
30. Katze, M. G., B. M. Detjen, B. Safer, and R. M. Krug. 1986. Translational control by influenza virus: suppression of the kinase that phosphorylates the alpha subunit of initiation factor eIF-2 and selective translation of influenza viral mRNAs. *Mol. Cell. Biol.* **6**:1741–1750.
31. Klessig, D. F. 1977. Isolation of a variant of human adenovirus serotype 2 that multiplies efficiently on monkey cells. *J. Virol.* **21**:1243–1246.
32. Klessig, D. F., and C. W. Anderson. 1975. Block to multiplication of adenovirus serotype 2 in monkey cells. *J. Virol.* **16**:1650–1688.
33. 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.
34. 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.
35. Kozak, M. 1986. Bifunctional messenger RNAs in eukaryotes. *Cell* **47**:481–483.
36. Kozak, M. 1988. Leader length and secondary structure modulate mRNA function under conditions of stress. *Mol. Cell. Biol.* **8**:2737–2744.
37. Leff, S. E., R. M. Evans, and M. G. Rosenfeld. 1987. Splice commitment dictates neuron-specific alternative RNA processing in calcitonin/CGRP gene expression. *Cell* **46**:517–524.
38. Lenk, R., and S. Penman. 1979. The cytoskeletal framework and poliovirus metabolism. *Cell* **16**:289–301.
39. Levine, B. J., N. Chodchoy, W. F. Marzluff, and A. I. Skoultschi. 1987. Coupling of replication type histone mRNA levels to DNA synthesis requires the stem-loop sequence at the 3' end of the mRNA. *Proc. Natl. Acad. Sci. USA* **84**:6189–6193.
40. Lizardi, P. M., V. Mahdavi, D. Shields, and G. Candelas. 1979. Discontinuous translation of silk fibroin in a reticulocyte cell-free system and in intact silk gland cells. *Proc. Natl. Acad. Sci. USA* **76**:6211–6215.
41. Lodish, H. F. 1974. Model for the regulation of mRNA translation applied to haemoglobin synthesis. *Nature (London)* **251**:385–388.
42. Logan, J., and T. Shenk. 1984. Adenovirus tripartite leader sequences enhance translation of mRNAs late after infection. *Proc. Natl. Acad. Sci. USA* **81**:3655–3659.
43. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory. Cold Spring Harbor, New York.
44. Mansour, S. L., T. Grodzicker, and R. Tjian. 1986. Downstream sequences affect transcription initiation from the adenovirus major late promoter. *Mol. Cell. Biol.* **6**:2684–2694.
45. Meyuhos, O., E. A. Thompson, and R. P. Perry. 1987. Glucocorticoids selectively inhibit translation of ribosomal protein mRNAs in P1798 lymphosarcoma cells. *Mol. Cell. Biol.* **7**:2691–2699.
46. Moore, M., J. Schaak, S. B. Baim, R. I. Morimoto, and T. Shenk. 1987. Induced heat shock mRNAs escape the nucleoplasmic transport block in adenovirus-infected HeLa cells. *Mol. Cell. Biol.* **7**:4505–4512.
47. Nevins, J. R., and M. J. Wilson. 1981. Regulation of adenovirus 2 gene expression at the level of transcriptional termination and RNA processing. *Nature (London)* **290**:113–118.
48. Ornelles, D. A., E. G. Fey, and S. Penman. 1986. Cytochalasin releases mRNA from the cytoskeletal framework and inhibits protein synthesis. *Mol. Cell. Biol.* **6**:1650–1662.
49. Pachter, J. S., T. J. Yen, and D. W. Cleveland. 1987. Autoregulation of tubulin expression is achieved through specific degradation of polysomal tubulin mRNAs. *Cell* **51**:283–292.
50. Palmiter, R. D. 1972. Regulation of protein synthesis in chick oviduct. *J. Biol. Chem.* **247**:6770–6780.
51. Pandey, N. B., and W. F. Marzluff. 1987. The stem-loop structure at the 3' end of histone mRNA is necessary and sufficient for regulation of histone mRNA stability. *Mol. Cell. Biol.* **7**:4557–4559.
52. Pelletier, J., and N. Sonenberg. 1988. Internal initiation of translation of eukaryotic mRNA directed by a sequence derived from poliovirus RNA. *Nature (London)* **334**:320–324.
53. Pilder, S., M. Moore, J. Logan, and T. Shenk. 1986. The adenovirus E1B-55K transforming polypeptide modulates transport or cytoplasmic stabilization of viral and host cell mRNAs. *Mol. Cell. Biol.* **6**:470–476.
54. Protzel, A., and A. J. Morris. 1974. Gel chromatographic analysis of nascent globin chains. *J. Biol. Chem.* **249**:4594–4600.
55. Quinlan, M. P., and D. F. Klessig. 1982. Normal translation of human adenovirus in cell-free lysates prepared from abortively as well as productively infected monkey cells. *J. Virol.* **44**:426–430.
56. Rao, C. D., M. Pech, K. C. Robbins, and S. A. Aaronson. 1988. The 5' untranslated sequence of the c-sis/platelet-derived growth factor 2 transcript is a potent translational inhibitor. *Mol. Cell. Biol.* **8**:284–292.
57. Richardson, W. D., and C. W. Anderson. 1984. Translation of adenovirus 2 late mRNAs microinjected into cultured African green monkey kidney cells. *J. Virol.* **51**:559–562.
58. Roper, M. D., and W. D. Wicks. 1978. Evidence for acceleration of the rate of elongation of tyrosine aminotransferase nascent chains by dibutyl cyclic AMP. *Proc. Natl. Acad. Sci. USA* **75**:140–144.
59. Schneider, R. J., C. Weinberger, and T. Shenk. 1984. Adenovirus VAI facilitates the initiation of translation in virus-infected cells. *Cell* **37**:291–298.
60. Sleat, D. E., D. R. Gallie, R. A. Jefferson, M. W. Bevan, P. C. Turner, and T. M. A. Wilson. 1987. Characterization of the 5' leader sequence of tobacco mosaic virus RNA as a general enhancer of translation in vitro. *Gene* **271**:217–225.
61. Theodorakis, N. G., S. S. Banerji, and R. I. Morimoto. 1988. HSP70 mRNA translation in chicken reticulocytes is regulated at the level of elongation. *J. Biol. Chem.* **263**:14579–14585.
62. Theodorakis, N. G., and R. I. Morimoto. 1987. Posttranscriptional regulation of hsp 70 expression in human cells: effect of heat shock, inhibition of protein synthesis, and adenovirus infection on translation and mRNA stability. *Mol. Cell. Biol.* **7**:4357–4368.
63. Trempe, J. B., and B. J. Carter. 1988. Alternative splicing is required for synthesis of adeno-associated virus VP1 capsid protein. *J. Virol.* **62**:3356–3363.
64. Uhlen, M., C. Svensson, S. Josephson, P. Alestrom, J. B. Chattopadhyaya, U. Pettersson, and L. Philipson. 1982. Leader arrangement in the adenovirus fiber mRNA. *EMBO J.* **1**:249–254.
65. Van der Vliet, P. C., and A. J. Levine. 1973. DNA binding proteins specific for cells infected by adenovirus. *Nature (London)* **246**:170–173.
66. Wilhelm, J. M., and H. S. Ginsberg. 1972. Synthesis in vitro of type 5 adenovirus capsid proteins. *J. Virol.* **9**:973–980.
67. Wolin, S. L., and P. Walter. 1988. Ribosome pausing and stacking during translation of a eukaryotic mRNA. *EMBO J.* **7**:3559–3569.
68. Wong, E. A., and I. E. Scheffler. 1982. Temperature-sensitive Chinese hamster fibroblast mutant with a defect in RNA metabolism. *Mol. Cell. Biol.* **2**:1558–1573.
69. Yager, D. R., and D. M. Coen. 1988. Analysis of the transcript of the herpes simplex virus DNA polymerase gene provides evidence that polymerase expression is inefficient at the level of translation. *J. Virol.* **62**:2007–2015.
70. Zain, S., J. Sambrook, R. J. Roberts, W. Keller, M. Fried, and A. R. Dunn. 1979. Nucleotide sequence analysis of the leader segments in a cloned copy of adenovirus 2 fiber mRNA. *Cell* **16**:851–861.
71. Zorn, G. A., and C. W. Anderson. 1981. Adenovirus type 2 expresses fiber in monkey-human hybrids and reconstructed cells. *J. Virol.* **37**:759–776.