Adenovirus Late Protein Synthesis Is Resistant to the Inhibition of Translation Induced by Poliovirus*

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Inhibition of host protein synthesis after poliovirus infection has been suggested to be a consequence of the proteolytic degradation of a p220 polypeptide necessary to translate capped mRNAs. However, the synthesis of several adenovirus late proteins on capped mRNAs was resistant to poliovirus inhibition. Thus, the hexon protein was still made 8 h after poliovirus superinfection. The synthesis of other adenovirus proteins, such as the fiber, was much more sensitive to poliovirus-induced inhibition than the hexon, either in the absence or in the presence of guanidine. Detailed densitometric analyses clearly showed the differential behavior of several adenovirus late mRNAs to poliovirus shut-off of translation. This is striking in view of the fact that a common leader sequence in the 5' termini is present in the adenovirus late mRNAs. The use of 3-methyl quercetin, an inhibitor of poliovirus RNA synthesis (Castrillo, J. L., Vanden Berghe, D., and Carrasco, L. (1986) Virology 152, 219-227), showed that translation of several capped adenovirus mRNAs took place in poliovirus-infected cells after the synthesis of host proteins had ceased. The poliovirus mRNA and the adenovirus mRNA coding for the hexon protein are very efficient mRNAs and have a leader sequence of more than 740 and 250 nucleotides, respectively, with very rich secondary structures making it difficult to predict how the scanning model will operate on these two mRNAs.

A drastic inhibition of host translation occurs soon after poliovirus infection of susceptible cells (for reviews, see 2-4). The mechanism involved in this inhibition remains unknown. Basically, two models have been put forward to account for this interference. One is that virus infection modifies the membrane, and this modification has consequences for cellular protein synthesis (3, 5). The other model suggests that during poliovirus replication a p220 polypeptide that forms part of the cap-binding complex is cleaved (2, 6, 7). Inactivation of this protein would selectively inhibit the translation of both cellular and viral capped mRNAs. Protein synthesis directed by poliovirus mRNA, which is uncapped, will not require a functional p220 protein (2, 4). Several in vitro observations support this view. Thus, cell-free systems obtained after poliovirus infection efficiently made proteins directed by uncapped mRNAs but did not translate capped mRNAs. This inactivation was reversed by addition of a cap-binding complex (8-10). However, several lines of evidence obtained from experiments with intact poliovirus-infected cells do not support the p220 inactivation model: 1) a poliovirus mutant which efficiently shuts-off translation in HeLa cells does not cleave the p220 protein (11). 2) No exact correlation exists between the cleavage of p220 and host cell shut-off (6). 3) Under some conditions, the synthesis of proteins on capped and uncapped mRNAs coexists in poliovirus-infected cells (13). 4) The inhibition of translation by poliovirus is dependent on viral gene expression, but none of the two known virus-coded proteases are involved in this inhibition (14-16). Therefore, more work is needed to elucidate the exact mechanisms by which poliovirus interferes with host protein synthesis.

Adenoviruses are also known to provoke a profound arrest of host translation late during their replication cycle (17). The transport and maturation of transcripts from the nucleus to the cytoplasm are blocked (18). However, this effect alone does not account for the inhibition of host translation, since cellular mRNAs remain present in a translatable form in the cytoplasm of adenovirus-infected cells (19, 20). Recent studies with adenovirus mutants unable to synthesize the small late VA RNAs (VA RNA I and II) have implicated these molecules in the regulation of translation (21). Recently evidence has accumulated suggesting that these small adenovirus VA RNAs block the phosphorylation of the initiation factor eIF2 (22, 23). In the absence of VA RNAs this factor becomes phosphorylated and neither viral nor cellular protein synthesis takes place.

The ability of ribosomes and factors to initiate translation on mRNAs depends on several conditions. One aspect that has received much attention recently is the secondary structure of the leader sequence present in mRNA preceding the AUG initiation codon (24). In principle, mRNAs with abundant secondary structure in their leader sequence should be translated less efficiently than others with less elaborated structures (25, 26). Cap-binding proteins are thought to participate in the melting of this secondary structure in an ATP-dependent process (4). The use of cells doubly infected with two different viruses doubtless helps to elucidate the mechanisms used by animal viruses to block cellular translation. In this study we provide evidence that adenovirus late protein synthesis is resistant to the inhibition of translation induced by poliovirus. These results agree well with the findings that the adenovirus late tripartite leader sequence confers efficient translation when ligated to another viral or cellular mRNA (27, 28).

MATERIALS AND METHODS

Cells and Virus—HeLa and BHK-21 cells were propagated in Petri dishes (Falcon Plastics) containing 10 ml of Eagle's medium as

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Fig. 1. Kinetics of protein synthesis in HeLa cells infected with adenovirus (○), poliovirus (●), or adenovirus and superinfected with poliovirus at 16 h postinfection (★). The multiplicity of infection was 10 plaque-forming units/cell for both viruses. Panel A, control without guanidine (–GND). Panel B, plus 3 mM guanidine (+GND). Cells were labeled with [35S]methionine and the proteins analyzed as described under “Materials and Methods.” The times refer to poliovirus infection.

modified by Dulbecco (E4D), supplemented with 10% newborn calf serum (Gibco), and incubated at 37°C in a 5% CO2 atmosphere.

Poliovirus type 1 (Mahoney strain) and adenovirus type 5 were grown on HeLa cells in E4D medium supplemented with 2% newborn calf serum (E4D2). Vesicular stomatitis virus (VSV) was grown on BHK-21 cells in the same medium. Cells and medium were collected and sedimented at 4000 rpm for 20 min. The pellet was resuspended in distilled water, freeze-thawed three times, and centrifuged at 4000 rpm for 15 min. The supernatants of both centrifugations were mixed and the concentration of virus estimated by plaque assay.

Conditions of Infection—HeLa cells grown in 24-well Linbro plates were infected with virus at the multiplicity of infection described in each experiment. After 30 min of incubation at 37°C, the medium was removed, and then 0.2 ml of Eagle’s medium as modified by Dulbecco supplemented with 2% newborn calf serum (E4D2) was added. The time of virus addition was considered as minus 30 min and zero time was taken as the point when the virus was removed. Incubation of the infected cells at 37°C was continued until the labeling period indicated in each experiment.

Analysis of Proteins by Polyacrylamide Gel Electrophoresis—At various times after infection, as indicated under “Results.” 6.2 ml of methionine-free E4D2 medium and 5 μCi of [35S]methionine (1450 Ci/mmol; The Radiochemical Centre, Amersham Corp.) were added to the cells and labeling allowed to proceed for 1 h. At the end of the labeling periods, the cell monolayers were dissolved in 200 μl of sample buffer (62.5 mM Tris, pH 6.8, 2% sodium dodecyl sulfate, 0.1 M dithiothreitol, 1% glycerol, and 0.024% bromphenol blue as indicator). Each sample was sonicated to reduce viscosity and heated at 90°C for 5 min. Volumes of 5 μl were applied to a 15% polyacrylamide gel and electrophoresed overnight at 100 V.

Fluorography of the gel was carried out with 2,5-diphenyloxazole dissolved in dimethyl sulfoxide (20% w/v). The gels were placed in a microwave oven (Sharp-R-8000E) and dried in only 6 min as described previously (1). The gels were exposed using RP-X-ray films (LKB). Densitometric profiles of the gel were analyzed using a Joyce Loeb Chromoscan 3 microdensitometer.

Aliquots of 5 μl of each sample were also precipitated by 1 ml of 10% trichloroacetic acid and filtered through GF/C glass fiber filters to determine the total protein synthesized. Dried filters were counted in an LKB-1219-Rackbeta liquid scintillation counter.

Source of Inhibitors—Guanidine-HCl (Aminomethanamidine-HCl) was from Sigma. 3MQ (5,7,3',4'-tetrahydroxy-3-methoxyflavone) a new inhibitor of poliovirus RNA synthesis (1) was kindly given to us by Dr. Vanden Bergh (Belgium).

Fig. 2. Electrophoretic analysis of the proteins synthesized in HeLa cells infected with adenovirus and superinfected with poliovirus after 16 h. Cells were treated or not with 3 mM guanidine at zero time (see scheme). The proteins synthesized in adenovirus- (II, p100-IX) and poliovirus- (I,3-2B) infected HeLa cells are indicated. The times refer to poliovirus infection.
poliovirus replication is blocked by guanidine (Fig. 1, panel B). However, no inhibition of total protein synthesis is observed over the first 5 h of poliovirus superinfection of adenovirus-infected cells (Fig. 1). Polyacrylamide gel electrophoresis analysis of the proteins synthesized in those cells indicates that synthesis of each late protein in control adenovirus-infected cells does not follow the same kinetics (Fig. 2). Thus, synthesis of proteins p100 and IIIa declines from the 20th hour following adenovirus infection, whereas synthesis of other late proteins, such as II (hexon), IV (fiber), or VII continues to be synthesized 23 h postinfection. It must be kept in mind that the synthesis of adenovirus late mRNAs follows a complicated splicing mechanism, giving rise to five adenovirus late mRNA families (33, 34). These mRNAs share regions of the leader sequence present in their 5′ region (34, 35), and all contain capped 5′ termini (36). Translation efficiency depends chiefly upon the secondary structure of mRNA 5′ terminus (26). Therefore, the differences in the leader sequences of mRNAs coding for proteins p100-IIIa and those coding for proteins II (hexon), IV (fiber), and VII can provide a structural basis for the different translational behavior observed with these mRNAs.

Superinfection of adenovirus-infected HeLa cells with poliovirus 16 h postinfection shows that poliovirus protein synthesis is detectable after the 3rd or 4th hour of poliovirus infection, is maximal between the 5th and 6th hour, then declines during the 7th and 8th hour. The synthesis of some adenovirus proteins, for example, pIV (fiber), is sensitive to poliovirus infection. Strikingly, synthesis of protein II (hexon) takes place during poliovirus translation and continues even during the 8th hour postinfection (Fig. 2). The presence of the sufficient concentration of guanidine that blocks poliovirus RNA synthesis but not the shut-off of host translation (37) demonstrates the continued synthesis of the hexon protein during the poliovirus replication cycle (Fig. 2). Under these conditions synthesis of the fiber was again much more sensitive to inhibition than other adenovirus late proteins. The fact that mRNAs coding for the fiber and hexon show a different behavior despite sharing a common sequence at their 5′ termini is very striking (34).

Measurement of individual proteins by densitometry clearly shows that synthesis of adenovirus hexon protein, pVII, follows an entirely distinct course than the synthesis of cellular actin, or adenovirus fiber in cells doubly infected with adenovirus and poliovirus, either in the presence or absence of guanidine (Fig. 3).

The rate and extent of poliovirus-induced shut-down of host translation depends primarily on the multiplicity of infection employed (38). The effect of poliovirus multiplicity of infection on the inhibition of adenovirus late protein synthesis is shown in Fig. 4. Synthesis of the cellular protein actin clearly decreases between 3–4 h postinfection, as the multiplicity of infection of poliovirus increases. Virtually no actin synthesis is detected when 40 plaque-forming units/cell of poliovirus are used. Strikingly, almost no inhibition in the synthesis of the hexon protein is observed, particularly when guanidine is present (Fig. 4). These data reinforce the view that adenovirus late protein synthesis and poliovirus translation do coexist even after cellular protein synthesis has ceased.

Recently, a new inhibitor of poliovirus replication has been described, 3-methyl-quercetin (3 MQ) (1). This compound efficiently blocks poliovirus RNA synthesis without affecting the kinetics of inhibition of cellular protein synthesis. Adenovirus translation is not affected by 3-MQ (Fig. 5). Moreover, the synthesis of several late adenovirus proteins continues after poliovirus infection when this inhibitor is added at various times, even though cellular translation gradually de-
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Fig. 4. Electrophoretic analysis of the proteins synthesized in HeLa cells infected with adenovirus (10 plaque forming units/cell) and superinfected with different multiplicities of infection of poliovirus after 16 h. 3 mM guanidine, was added (+) or not (−) at zero time. Cells were labeled with [35S]methionine during 3-4 h after poliovirus infection. The proteins were analyzed as described under “Materials and Methods.”

Enzymatic quantitation of individual proteins clearly illustrates that actin synthesis is inhibited 90% 5 h postinfection, whereas substantial amounts of the hexon proteins are still being made at that time either in the presence or absence of 3 MQ (Fig. 6).

DISCUSSION

The regulation of translation in mammalian cells plays an important part in gene expression. The ability of mRNAs to bind and efficiently compete for certain components of the translation machinery must be crucial for the final outcome of protein synthesized by a particular mRNA (24). The structure of the mRNA is determinant for its affinity to bind to initiation complexes (24). According to the “scanning model,” these initiation complexes bind to regions near the 5′ end of mRNA and travel through the mRNA until an initiation codon is reached (39). The initiation codon is recognized by the primary structure of mRNA surrounding it. mRNAs with regions rich in secondary structure upstream of the initiation codon are less efficiently translated than those with simpler
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**FIG. 5.** Electrophoretic analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the proteins synthesized in HeLa cells superinfected with adenovirus and poliovirus (see scheme). The cells were treated or not with 3MQ (20 μg/ml) at zero time or 2 h postinfection. The multiplicity of infection was 10 plaque-forming units/cell for both viruses. Cells were labeled with [35S]methionine and the proteins analyzed as described under "Materials and Methods." The times refer to poliovirus infection.

and shorter sequences between the 5′ end and the initiation codon (25, 26). Cap-binding factors and perhaps other components involved in initiation may be responsible for melting those sequences when initiation complexes travel through these noncoding sequences of the mRNAs (4, 24). Ionic conditions influence the stability of these secondary structures (26, 40). Thus, hypertonicity stabilizes hydrogen bonds and inhibits the melting and subsequent translation of those mRNAs with greater secondary structures in their 5′ end (26). The picornavirus mRNA has the initiation codon more than 700 nucleotides from the 5′ end (41). In spite of the possibility of a rich secondary structure in this region, this mRNA is the most resistant mammalian mRNA to hypertonic conditions (42, 43). In fact, its translation is optimal under hypertonic conditions (44) suggesting that internal initiation could occur (45). This is particularly evident when the computer-predicted secondary structure of the 5′ region is observed. At least two very stable hairpins appear, one between nucleotides 230 and 328 (ΔG° = -55 Kcal·mol⁻¹), and another between nucleotides 349 and 406 (ΔG° = -39 Kcal·mol⁻¹). Preceding from the
than poliovirus translation (13). Moreover, complete removal of the 5'-untranslated regions of several adenovirus early mRNAs does not alter their translation efficiency (47). These results suggest the possibility that internal initiation could also occur on adenovirus mRNAs.

Although, cell-free systems from poliovirus-infected cells do not translate capped viral nor cellular mRNAs (see reviews; 2 and 4), some capped viral mRNAs are translated provided that the secondary structure is destroyed by melting (12). The idea behind these experiments is that cap-binding proteins are involved in the melting of secondary regions in the 5'-noncoding sequence of mRNAs. Since the cap-binding complex is not functional in poliovirus-infected cell extracts, these mRNAs are not translated in these systems. Addition of more cap-binding factors or melting of these secondary structures overcomes this inhibition (4). Our finding that late adenovirus mRNAs with long and possibly highly structured 5'-noncoding regions are translated in poliovirus-infected cells and are efficient mRNAs as defined by their resistance to hypertonic medium (46) suggests that the mRNA melting model might not be of universal applicability.

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REFERENCES

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