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Reovirus-induced modification of cap-dependent translation in infected L cells

(*in vitro* translation/reoviridae/translational control)

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ABSTRACT **The translational apparatus in cell-free extracts prepared from L cells infected with reovirus undergoes a time-dependent transition from cap dependence to cap independence. Extracts from uninfected L cells translate capped reovirus mRNA at high efficiency and synthesize the expected three size classes of reovirus polypeptides, and the translation is sensitive to $m^7G(5')ppp$. This same extract translates uncapped mRNA at a much lower efficiency. In contrast, extracts from infected L cells translate uncapped reovirus mRNA at high efficiency and synthesize the correct three size classes of polypeptides, and the translation is not sensitive to inhibition by $m^7G(5')ppp$. Infected cell extracts translate capped mRNA at reduced efficiency (≈25%), the translation is not sensitive to inhibition by $m^7G(5')ppp$, and the correct three size classes of viral polypeptides are not synthesized. These observations may explain how reovirus takes over the host translational apparatus.**

A wide variety of cellular and viral mRNAs contain the capped structure $m^7G(5')ppp(5')Np^m$ at their 5′ ends (1). Only a few nonprokaryotic mRNAs lack capped 5′ ends; e.g., poliovirus (2), encephalomyocarditis virus†, satellite tobacco necrosis virus (3), and cow-pea mosaic virus (4). These observations have led to the idea that all eukaryote and the vast majority of viral mRNAs contain a capped structure at their 5′ terminus.

Considerable information has accumulated on the role of the capping group in translation, and what was at first thought to be a clear picture has steadily become more complicated (5). Earlier reports suggested that caps are required for translation of vesicular stomatitis virus and reovirus mRNA in the wheat germ cell-free, protein-synthesizing system (6), and a similar picture seemed to be emerging for rabbit globin mRNA (7). This picture has become complicated by the finding that the degree of dependence on caps could vary widely. For example, the mRNAs of poliovirus and encephalomyocarditis virus, which are not capped in their natural state, are translated by *in vitro* protein-synthesizing systems (8, 9). Further, *in vitro* translation of brome mosaic virus RNA-4 (10) and vesicular stomatitis virus mRNA (11, 12) is only partially impaired by removal of the capping group. So although it is clear that the cap structure is important for translation of eukaryotic mRNA, it is not possible to make any generalized statement as to what this role might be.

In L cells infected with reovirus the overall rate of protein synthesis remains high for a considerable period. Synthesis of reovirus-specific proteins starts very slowly, but by the time late viral mRNAs are being transcribed, virus-specific proteins are being synthesized almost to the exclusion of host proteins (13). The mechanism of this takeover is unknown. We have examined the possible role of changes in the methylation pattern of the viral mRNA in this process. The recent development of a method for making efficient cell-free protein-synthesizing systems of extremely low background activity from L cells (14) has enabled us to compare the *in vitro* translation of exogenously supplied reovirus mRNA in extracts that have been prepared from infected and uninfected L cells. Using extracts from infected cells, we found that the translation of uncapped reovirus mRNA is highly efficient and that the ability of these extracts to translate this form of reovirus mRNA increases with time of infection. Our observations lead us to conclude that the host cell translational machinery becomes modified as a result of viral infection. A model is proposed for the reovirus-induced takeover of the host cell translation machinery, in which uncapped viral mRNAs are preferentially translated at late times during infection.

MATERIALS AND METHODS

Cells, Reovirus, and Reovirus mRNA. Reovirus, type 3 (Dearing strain) was grown in mouse L cells and purified as described (15). Reovirus mRNA was synthesized *in vitro* as described (16). Radioactive precursors were added or not according to the needs of the experiment. For preparing labeled mRNA, the concentration of the corresponding triphosphate was lowered to 0.4 mM and the radioactive triphosphate (New England Nuclear) was added. For [β-^{32}P]GTP, this was prepared by the method of Furuichi and Shatkin (17) and added to a final specific activity of 1–3 Ci/mmol (1 Ci = 3.7×10^{10} becquerels). The reaction mixture for making capped mRNA contained 10 μM *S*-adenosylmethionine; that for uncapped mRNA contained 320 μM *S*-adenosylhomocysteine (AdoHcy) and 0.5 mM pyrophosphate. Under the latter conditions, the mRNA synthesized is composed predominantly (98%) of the structure ppGp . . . at the 5′ end (18).

Reaction mixtures for preparing mRNA with blocked but unmethylated 5′ termini contained 320 μM AdoHcy but no pyrophosphate. Under these conditions, 75% of the 5′ termini of the mRNA synthesized are composed of the structure GpppGp. . . , the remaining 25% has the structure ppGp (18). These structures were confirmed by examination of the structure of RNA labeled at the 5′ end with [β-^{32}P]GTP. mRNA was purified as described (14).

Standard System for *In Vitro* Protein Synthesis. Preparation of S-10 extracts and treatment of these extracts with micrococcal nuclease have been described (14). *In vitro* protein synthesis was carried out in a final volume of 50 μl containing the following components: 30 μl of nuclease-treated S-10 extract, 1 mM ATP (neutralized with KOH), 10 mM creatine phosphate, 1.2 mg of creatine phosphokinase (EC 2.7.3.2) per

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Abbreviation: AdoHcy, *S*-adenosylhomocysteine.

† Fellner, P., Frisby, D., Goodchild, J., Porter, A. & Carey, N. H. (1975) *3rd International Congress Virol.*, Abstr. 161.

ml, 30 mM Hepes (pH 7.5), 90 mM KCl (unless otherwise specified), 4 mM $Mg(OAc)_2$, 1 mM dithiothreitol, 0.5 mM GTP, 5μM of each amino acid in a mixture lacking methionine, 800 μCi of [^{35}S]methionine per ml (New England Nuclear, specific activitiy 380–460 Ci/mmol), 7 pmol of reovirus mRNA, and water or other additions to a final volume of 50 μl. Incubation was carried out at 30°C for the times indicated. Unless otherwise specified, 5-μl aliquots were removed and spotted on Whatman 3 MM filter paper discs and washed for 10 min in ice-cold 10% trichloroacetic acid containing unlabeled methionine. The filters were transferred to hot (90°C) 5% trichloroacetic acid for another 10 min, then washed twice in ice-cold 5% trichloroacetic acid, once in ethanol, once in ethanol/ether, 3:1 (vol/vol), and finally in ether alone. Filters were dried and placed in 5 ml of toluene-base scintillation fluid; radioactivity was determined in an Intertechnique SL30 liquid scintillation counter.

The remaining portion of the incubation mixture was made 10% trichloroacetic acid/3% Casamino acids and placed on ice for 10 min. The pellet, obtained by centrifugation, was suspended in 5% trichloroacetic acid/3% Casamino acids, heated at 90°C for 10 min, centrifuged again, washed three times with 5% trichloroacetic acid containing Casamino acids, once with acetone, dried, and dissolved in sample buffer for analysis by polyacrylamide gel electrophoresis. Polyacrylamide gel electrophoresis was done according to Laemmli (19). Radioautography was done as described (20). Except where indicated, all S-10 extracts from infected cells were prepared from L cells that had been infected with reovirus at a multiplicity of 5 plaque-forming units/cell and incubated for 30 hr at 31°C. Under these conditions, the infected cells showed no signs of viral induced cytopathic effects, and >95% excluded trypan blue.

RESULTS

Translation of Capped and Uncapped Reovirus mRNA in Uninfected and Infected L-Cell Extracts. In a previous study, involving translation of reovirus mRNA in a protein-synthesizing system derived from wheat germ (6), Both *et al.* observed that reovirus mRNA, which contains the 5′-terminal cap $m^7G(5')ppp(5')Gm\ldots$, was translated with high efficiency whereas uncapped mRNA, terminating with the nucleoside diphosphate ppG. . ., was translated to only a small extent, if at all. This striking difference in the *in vivo* translational capacity of capped and uncapped mRNA was confirmed in the present study with extracts prepared from normal, uninfected L cells (Fig. 1). However, when samples of capped and uncapped reovirus mRNAs were translated under identical conditions, but in extracts prepared from reovirus-infected cells, a different result was obtained. As Fig. 1 demonstrates, reovirus mRNA that lacked the capping group was translated with high efficiency in extracts from infected cells. Moreover, the rate of translation of this mRNA in these extracts was virtually identical to that obtained when capped mRNA was translated with extract of normal, uninfected cells. Uncapped mRNA is not translated in extracts prepared from normal L cells; thus, this constitutes a major difference in the behavior of the two systems. In this series of experiments capped mRNA was translated only 25% as efficiently in infected-cell extracts as compared either with its translation in normal extracts or with the translation of uncapped mRNA in extracts from infected cells. It is evident from these results that significant changes relating to the cap requirement for translation occur during reovirus infection. The uncapped mRNA used in this part of the study was composed predominantly of the structure ppGp. . . at the 5′ end. It is conceivable that this mRNA, when added to an S-10 extract, might condense with GTP to form the structure $G(5')ppp(5')Gp\ldots$, but it could not be methylated

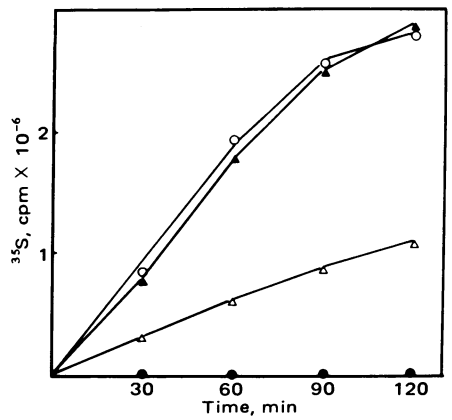

FIG. 1. Kinetics of incorporation of [^{35}S]methionine. Standard S-10 extracts were prepared from uninfected cells and from cells that had been infected by reovirus for 30 hr at 31°C. Each S-10 extract was primed with capped or uncapped reovirus mRNA in the standard system and incubated at 30°C for the times indicated. At each time point, 5 μl of reaction mixture was spotted onto filters and assayed for hot trichloroacetic acid-precipitable radioactivity. ● and ▲, Uninfected extracts; ○ and △, infected extracts. △ and ▲, Priming with capped mRNA; ○ and ●, priming with uncapped mRNA.

in the presence of AdoHcy. Moreover, mRNA with the structure GpppGp translated poorly in extracts from uninfected and infected cells in the presence of AdoHcy (data not shown).

Stability of mRNA Under Translating Conditions. In order to properly assess the relevance of the differences between the behavior of extracts from normal and reovirus-infected L cells in relation to translation of capped and uncapped mRNA, it was important to see whether incubation under translating conditions had any effect on the structure of the mRNA used in the experiments. In order to get some insight into this we examined the stability of RNA double labeled with [β-^{32}P]GTP and [^{3}H]CTP. The former compound specifically labels the 5′ end of the RNA molecules, whereas the latter provides a marker for internal sequences. The structures of the capped and uncapped mRNA are, respectively, $m7Gppp\overset{*}{G}{}^mp\ldots[^3H]Cp\ldots C_{OH}$ and $\overset{*}{p}pGp\ldots[^3H]Cp\ldots C_{OH}$. These two types of mRNA were incubated under translating conditions in extracts from normal or reovirus-infected cells, and the amount of acid-precipitable radioactivity was determined at different times. AdoHcy was added to prevent capping in the extracts.

Fig. 2*A* shows the effect of incubation on mRNA in extracts from uninfected cells. Both the 5′-terminal and internal labels of capped mRNA were stable throughout the 90 min of incubation. In contrast, uncapped mRNA degraded rapidly to acid-soluble material with a half-life of ≈90 min. When AdoHcy was omitted from the incubation mixture, uncapped mRNA was protected from nucleolytic digestion, indicating the presence of capping enzymes in extracts from uninfected cells (data not shown). Similar results have been reported by others (21–23).

Fig. 2*B* shows the effect of incubation on capped and uncapped mRNA in extracts prepared from infected cells. Stability of capped mRNA was similar to that observed in extracts from uninfected cells (Fig. 2*A*). This is not the case for uncapped, ppGp. . .-terminated mRNA, which lost 80% of the 5′-terminal ^{32}P label during the first 10 min of incubation. On the other hand, the internal sequences, represented by the ^{3}H label, were stable throughout the incubation period. Subsequent extraction of the ^{3}H-labeled, uncapped mRNA, followed by analysis by velocity sedimentation in sucrose, showed that the ^{3}H label still sedimented as the typical three size classes of reovirus mRNA. No ^{32}P label sedimented in this region of the gradient. Structural analysis showed that when uncapped

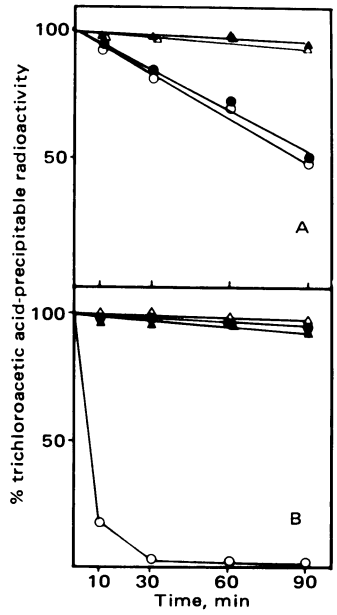

FIG. 2. Stability of capped and uncapped mRNA in extracts from uninfected (*A*) and infected (*B*) L cells. Capped and uncapped mRNAs having the structures m7G(5′)p^{32}pp(5′)-G^{m}p. . .[^{3}H]CMP. . .C_{OH} and ^{32}ppGp. . .[^{3}H]CMP. . .C_{OH}, respectively, were incubated in S-10 extracts under standard translation conditions. Aliquots were removed at the times indicated and assayed for acid-precipitable radioactivity. △ and ▲, Capped mRNA; ○ and ●, uncapped mRNA; ▲ and ●, ^{3}H; △ and ○, ^{32}P. All samples contained 96,000 cpm of ^{3}H and 10,000 cpm of ^{32}P.

mRNA was prepared by using [α-^{32}P]CTP and incubated as described above, the 5′-terminal structure was reduced from ppGpC. . . to pGpC. . . (data not shown).

Effect of m^{7}G(5′)ppp on *In Vitro* Translation. Translation of the two forms of reovirus mRNA in infected cell extracts was investigated further by measuring the effect of m^{7}G(5′)ppp on the rate of *in vitro* translation. Hickey *et al.* (3) and others (24–26) have observed that the translation of capped mRNA in several systems is inhibited by cap analogues such as m^{7}G(5′)ppp. Based on these inhibition studies, it was suggested that translation of capped mRNAs is dependent on the capping group. The translation of RNAs that are naturally uncapped was not affected by m^{7}G(5′)ppp in similar inhibition studies (3). In the present study, m^{7}G(5′)ppp was found to inhibit the translation of capped reovirus mRNA in extracts of normal, uninfected L cells by greater than 90% (Table 1). In direct constrast to this result, when extracts from infected cells were used, m^{7}G(5′)ppp affected the translation of neither the uncapped form nor the capped form of reovirus mRNA. This result points to another major difference in the properties of extracts derived from uninfected compared to infected L cells. Whereas normal extracts translated capped reovirus mRNA by a mechanism that is highly sensitive to inhibition by m^{7}G-(5′)ppp, the translation of either capped or uncapped reovirus mRNA in infected cell extracts apparently occurs by a mechanism that is insensitive to inhibition by m^{7}G(5′)ppp and, therefore, independent of the capping group.

Table 1. Inhibition of protein synthesis by m^{7}G(5′)ppp*

Extract	mRNA	m^{7}G(5′)ppp	cpm	% inhibition
Uninfected	—	—	109	
	—	+	129	
	Capped	—	844,832	—
	Capped	+	78,632	91
	Uncapped	—	8,448	—
	Uncapped	+	8,571	0
Infected	—	—	175	
	—	+	189	
	Capped	—	331,190	—
	Capped	+	342,681	0
	Uncapped	—	798,371	—
	Uncapped	+	801,231	0

* All reaction mixtures contained 320 μM AdoHcy. Concentration of m^{7}G(5′)ppp was 1 mM. Standard reactions were carried out for 90 min.

Conversion from Cap-Dependent to Cap-Independent Translation in Infected-Cell Extracts. Any mechanism proposed to explain the reovirus-induced shutoff of host cell protein synthesis must take into account the fact that, rather than occurring immediately after infection as is the case in some other viral systems, this phenomenon occurs gradually in cells infected by reovirus. Because caps could play a role in the mechanism of this virus-induced takeover of host cell protein synthesis, it was of interest to determine the onset of the ability of infected-cell extracts to support the translation of uncapped viral mRNA. As illustrated in Fig. 3, the translation of uncapped mRNA in infected cell extracts could be detected by 10 hr after infection at 31°C. Thereafter, the relative efficiency of translation of uncapped mRNA in these cell extracts increased steadily. Concomitant with this increase in the relative efficiency of translation of uncapped mRNA, there was a steady decline in the relative efficiency with which capped mRNA was translated in extracts from infected cells. By 30 hr after infection at 31°C, the relative rate of translation of capped mRNA dropped to ≈25% of its original value. Thus, the ability to translate uncapped mRNA in infected-cell extracts appears to arise gradually in the infected cell and is paralleled by a loss of cap-dependent translation.

Polyacrylamide Gel Electrophoretic Analysis of Polypeptides Synthesized *In Vitro*. Extracts prepared from uninfected and reovirus-infected L cells were primed with capped and uncapped reovirus mRNA and the polypeptides synthesized were examined by polyacrylamide gel electrophoresis. The results are shown in Fig. 4. Polypeptides synthesized in uninfected cell extracts primed with capped mRNA yielded the expected three size classes (Fig. 4, well A), corresponding to the three size classes of polypeptides present in purified virions (Fig. 4, well B). Extract prepared from infected cells and primed with uncapped mRNA also yielded polypeptides corresponding to those present in purified virions (Fig. 4, well C). In contrast to these results, infected cell extract primed with capped mRNA yielded a completely different pattern (Fig. 4, well D). In this case, the polypeptides ranged in size between the large (λ) size class to smaller than the small (σ) size class,

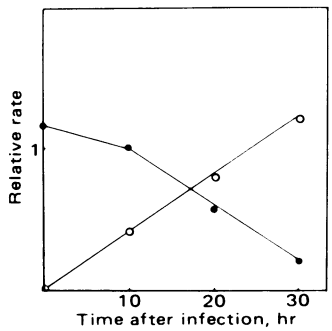

FIG. 3. Transition from cap-dependent to cap-independent translation in infected L cells. L cells were infected with reovirus at 31°C. At the times indicated, samples of cells were removed and S-10 extracts were prepared. Kinetics of incorporation of [^{35}S]methionine into host acid-precipitable material were determined for each point as in Fig. 1. Initial rates of protein synthesis were determined under conditions of saturating mRNA concentrations. The initial rates of incorporation of [^{35}S]methionine were 2.5 × 10^4 cpm/min with capped mRNA in S-10 extract prepared at time zero after infection and 2.8 × 10^4 cpm/min with uncapped mRNA in S-10 extract prepared at 30 hr after infection. ●, Translation of capped mRNA; ○, translation of uncapped mRNA.

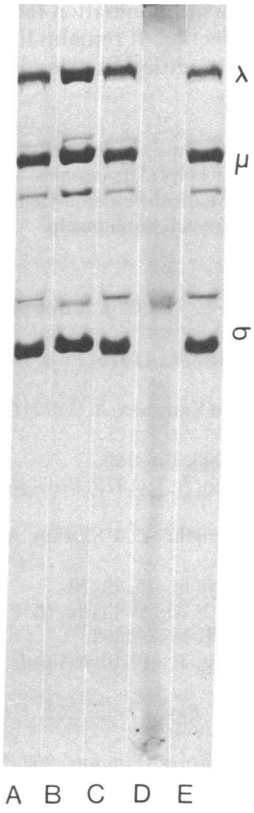

FIG. 4. Radioautogram of polyacrylamide gel electrophoresis of polypeptides synthesized *in vitro*. Standard protein-synthesizing reactions were performed. Electrophoresis was from top to bottom. Reaction mixtures contained: well A, extract from uninfected cells with 5 μg of capped reovirus mRNA; well B, reovirus marker proteins from purified virions; well C, extract from infected cells with 5 μg of uncapped mRNA; well D, extract from infected cells with 5 μg of capped mRNA; and well E, extract from infected cells with 5 μg of uncapped mRNA and 15 μg of capped mRNA.

with a prominent band in the σ region of the gel. This result showed that the infected-cell extract translated capped mRNA with less fidelity than it did uncapped mRNA. To investigate this phenomenon further, we primed infected-cell extract with an amount of uncapped mRNA corresponding to that used for the experiment analyzed in Fig. 4, well C, and, in addition, a 3-fold excess of capped mRNA was added. This experiment yielded the result shown in Fig. 4, well E, which clearly illustrates that the 3-fold excess of capped mRNA has no effect on the translation of uncapped mRNA. This means that the capped mRNA did not effectively compete with uncapped mRNA and suggests that high-affinity binding at the correct AUG in capped mRNA either does not occur or occurs at lower frequency in the infected-cell extract.

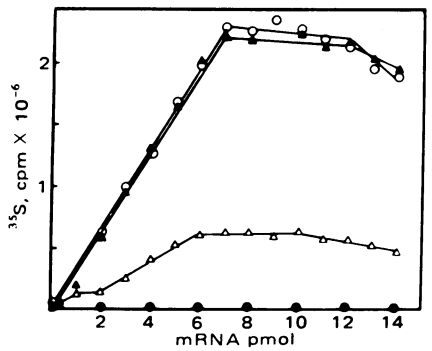

FIG. 5. Translation of capped and uncapped mRNAs in extracts from uninfected and infected cells as a function of mRNA concentration. Standard S-10 extracts were prepared from uninfected cells and from cells infected with reovirus for 30 hr at 31°C. Each S-10 extract was primed with capped or uncapped reovirus mRNA in the amounts indicated. Incubations were for 120 min at 30°C. Five microliters of reaction mixture were spotted onto filters and assayed for hot acid-precipitable radioactivity. ▲ and ●, Uninfected extracts; △ and ○, infected extracts. ▲ and △, Priming with capped mRNA; ●, and ○, priming with uncapped mRNA.

DISCUSSION

As reported by others, extracts prepared from uninfected cells for *in vitro* protein synthesis prefer capped reovirus mRNA to uncapped mRNA (6). The significance of the present paper lies in the observation that a transition occurs in the cap-dependent translational machinery as a result of virus infection (Fig. 1). Furthermore, this transition occurs gradually throughout the course of infection (Fig. 3).

In vitro translation of capped mRNA is more sensitive to changes in K^+ ion concentration than that of uncapped mRNA (27). Also, infection by picornaviruses such as encephalomyocarditis virus leads to changes in the cellular membrane, which in turn lead to an influx of Na^+ ions, and this change in monovalent cation concentration results in the differential translation of host and viral mRNAs (28, 29). Because the extracts used in the present study were not subjected to either gel filtration or dialysis, it was possible that different endogenous levels of ions might be effecting an apparent alteration of specificity. However, analysis of K^+ and Na^+ concentrations in the two types of extract and examination of a wide range of concentrations of these ions added to *in vitro* protein-synthesizing reactions, failed to support this idea (data not shown).

We have shown that extracts prepared from both infected and uninfected cells are clearly distinguishable in terms of their sensitivity to the capped analogue inhibitor $m^7G(5')ppp$ (Table 1). The lack of sensitivity of the infected-cell extracts to inhibition by $m^7G(5')ppp$ suggests that translation in the infected cell occurs by some mechanism that is independent of the capping group.

Although capped and uncapped mRNA of reovirus was used throughout these studies, we have also examined the behavior of globin mRNA in both types of extracts, as an example of *in vivo* synthesized capped mRNA (data not shown). Not unexpectedly, globin mRNA translated with high efficiency in extracts from uninfected cells and the synthesis was sensitive to $m^7G(5')ppp$. However, it translated at only ≈30% efficiency in extracts from infected cells, which corresponds to the 25% efficiency of translation of reovirus capped mRNA in similar extracts (Fig. 1). In addition, this 30% translation efficiency of globin mRNA was insensitive to $m^7G(5')ppp$ inhibition. Thus, globin mRNA has properties similar to those of reovirus capped mRNA in both uninfected and in infected cell extracts.

Polypeptides synthesized *in vitro* in an extract from uninfected cells with capped reovirus mRNA (Fig. 4, well A) have electrophoretic mobilities similar to those of the reovirus marker polypeptides (Fig. 4, well B). Extracts prepared from infected cells have the ability to translate uncapped mRNA at high efficiency (Fig. 1) and synthesize the normal three size classes of reovirus polypeptides (Fig. 4, well C). In direct contrast, capped mRNA is translated at reduced (about 25%) efficiency (Fig. 1). Although there is a possibility that this 25% residual activity is due to the presence of uncapped mRNA in the *in vitro* synthesized preparation of capped mRNA, this seems unlikely for two reasons. First, increasing the concentration of capped mRNA in the infected-cell extract did not increase the net synthesis of polypeptides. Instead, one finds that the differential translation of both capped and uncapped mRNA is evident over a wide range of mRNA concentrations (Fig. 5). Second, the polypeptides synthesized by using capped mRNA are not the normal reovirus polypeptides grouped into three size classes, but rather exist predominantly as a polypeptide migrating in the region of the σ size class (Fig. 4, well D). This observation raises the possibility that extracts from infected cells have lost their preferential, high-affinity binding for the 5′ termini of capped mRNAs, which would normally result in initiation at the correct AUG codon. In the absence of such affinity, internal initiations might occur with reduced efficiency at the exposed

AUG codons, leading to the pattern we see in Fig. 4, well D. The highly efficient translation of uncapped reovirus mRNA in these infected-cell extracts combined with the observation that these extracts synthesize the correct size classes of polypeptides (Fig. 4, well C) suggests that the correct initiation site is recognized under the condition of saturating mRNA concentrations used throughout this study. This hypothesis is supported by the observation that infected cell extracts, primed with a 3-fold excess of capped over uncapped mRNA (above saturating mRNA concentrations), synthesized predominantly full-length reovirus polypeptides (Fig. 4, well E). In this experiment, most of the radioactivity was incorporated into the three size classes of polypeptides, presumably directed by the uncapped mRNA (compare wells E and C). A negligible amount was incorporated into polypeptides migrating throughout the gel (compare wells E and D). We conclude from this experiment that uncapped mRNA was preferentially translated over capped mRNA in extracts prepared from infected cells. These data also suggest that recognition or affinity binding near the 5′ terminus either limits or prevents internal initiations in the mRNAs.

In L cells infected with reovirus, the synthesis of viral polypeptides begins slowly. As the infection progresses, there is a gradual increase in the translation of viral mRNA. Preferential synthesis of viral polypeptides *in vivo* begins somewhat before 20 hr after infection at 31°C (unpublished data) [or at the equivalent time of around 10 hr after infection at 37°C (13, 30)]. At late times the polypeptides made *in vivo* are exclusively viral (refs. 13 and 30 and unpublished data). These findings agree with the observed increase in preferential translation of uncapped mRNA in extracts prepared from infected L cells as a function of time after infection (Fig. 3). We therefore propose the following model to explain the takeover of protein synthesis in L cells infected with reovirus. Parental subviral particles produce single-stranded RNA copies of the double-stranded RNA genome. These single-stranded RNA transcripts serve as templates for the synthesis of a complementary strand in the production of progeny subviral particles (31). These single-stranded RNA transcripts are capped [as shown by the fact that the plus strand of the double-stranded RNA genome of virions is capped (32)] and, therefore, can be translated at early times by the normal cap-dependent translational apparatus of the host cell. Synthesis of viral mRNA at later times occurs predominantly from progeny subviral particles. Unpublished data from our laboratory indicates that (*i*) progeny subviral particles isolated from infected cells contain an active RNA polymerase activity and an *inactive* methyltransferase activity that can be activated by chymotrypsin, (*ii*) the single-stranded RNA products can be translated *in vitro* into reovirus polypeptides, and (*iii*) the 5′ end of the single-stranded RNA has the structure pGpCp. . . . The last finding is consistent with the present observation that [α-^{32}P]CTP-labeled, uncapped mRNA is rapidly reduced from a diphosphorylated to a monophosphorylated 5′ terminus during incubation in S-10 extracts from virus-infected cells. Additional unpublished data now clearly show that extensively purified reovirus mRNA synthesized *in vivo* at late times also terminates at the 5′ end with pGp. These data are consistent with our present hypothesis that mRNA synthesized by progeny subviral particles during infection is structurally different from that synthesized by the parental infecting virions and is preferentially translated by a modified host translational apparatus. We further suppose that uncapped mRNA synthesized by progeny subviral particles is not a template for minus-strand synthesis. In addition to taking over the host translational apparatus, such a mechanism would resolve an inherent conflict in translating a mRNA molecule that must also serve as a template for minus-strand synthesis. A recent report by Rose *et al.* (33) suggests that a translation initiation factor is modified as a result of poliovirus infection. It remains to be seen if a host initiation factor is also modified as a result of reovirus infection.

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