

# Inactivation of cap-binding proteins accompanies the shut-off of host protein synthesis by poliovirus

[*in vitro* translation/restoring activity/cap-binding protein inactivating factor(s)]

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Communicated by Aaron J. Shatkin, February 26, 1982

**ABSTRACT** Infection of HeLa cells with poliovirus results in a rapid shut-off of host protein synthesis. It has been suggested that inactivation of a protein that binds to the cap structure of cellular mRNAs would explain the selective inhibition of host protein synthesis because the naturally uncapped poliovirus RNA can be translated by a cap-independent mechanism. To test directly for the presence of cap-binding proteins in poliovirus-infected and mock-infected cells, we analyzed initiation factor preparations for their ability to specifically crosslink to the 5' cap structure of oxidized reovirus mRNA. The data presented here show that the crosslinking ability of the different cap-binding proteins (24-, 28-, 32-, 50-, and 80-kilodalton polypeptides) is reduced in preparations from poliovirus-infected as compared to mock-infected cells. This reduction correlates with the inability of initiation factor preparations from infected cells to restore translation of capped mRNAs in extracts of poliovirus-infected cells. In addition, initiation factor preparations from poliovirus-infected cells have the ability to rapidly inactivate cap-binding proteins and can also impair the restoring activity of initiation factors from mock-infected cells.

Viral infection of mammalian cells often results in the shut-off of host protein synthesis (1), and this effect has been extensively studied in poliovirus-infected HeLa cells (2, 3). Poliovirus infection neither induces the degradation of host mRNA (4, 5) nor causes detectable changes in the patterns of host mRNA capping, methylation, and polyadenylation (6). Furthermore, Ehrenfeld and Lund have demonstrated that host mRNA extracted from infected cells remains functional in a wheat-germ cell-free translation system (7).

It was established that the inhibition of host protein synthesis occurs at the initiation step (5), and subsequently it was shown that ribosomal high-salt-wash fractions from infected cells stimulated the translation of poliovirus mRNA, but not of endogenous mRNA, in HeLa cell extracts (8). Rose *et al.* (9), using vesicular stomatitis virus (VSV) mRNA as a model for host mRNAs (8, 10), and Helentjaris *et al.* (11) have presented evidence that suggested inactivation of eukaryotic initiation factors (IF) eIF-4B and eIF-3. However, a more recent study indicated that a 24-kilodalton (kDal) protein isolated by a multistep procedure (12) copurified with the ability to restore the capacity of poliovirus-infected HeLa cell extracts to efficiently translate VSV mRNA (this activity will be referred to as restoring activity) (12). This polypeptide was found to be identical to the 24-kDal cap-binding protein (24-CBP) isolated from rabbit reticulocytes by affinity chromatography on a column of 7-methylguanosine diphosphate ( $m^7GDP$ ) coupled to Sepharose 4B (13). The demonstrated copurification of 24-CBP with eIF-3 and eIF-4B (12, 14, 15) suggests that the effects ascribed to these factors were

due to the presence of the 24-CBP and that the shut-off phenomenon might actually be a result of inactivation of the 24-CBP.

Recently, Tahara *et al.* (16) described an 8-10S protein complex purified by  $m^7GDP$  affinity chromatography from rabbit reticulocyte ribosomal high-salt wash. This complex consisted of several higher molecular weight proteins in addition to the 24-CBP and possessed stable restoring activity. This finding is consistent with recent results demonstrating the existence of several higher molecular weight polypeptides that are structurally related to the 24-CBP of rabbit reticulocytes (17, 18). Moreover, some polypeptides with molecular weights that are strikingly similar to those of the latter polypeptides can specifically recognize the cap structure, as determined by crosslinking to oxidized mRNA (19).

In contrast to the almost ubiquitous nature of the cap structure at the 5' terminus of eukaryotic cellular and viral mRNAs, poliovirus RNA lacks a capped 5' end (20, 21) and its translation must therefore bypass any 5'-cap-dependent ribosome recognition mechanism. Inactivation of one or more of the CBPs would most likely result in a reduction of host mRNA translation and favor poliovirus RNA translation.

In an attempt to determine the fate of CBPs after poliovirus infection, we analyzed the ability of polypeptides in crude initiation factor preparations from poliovirus-infected and mock-infected cells to specifically crosslink to the 5' terminus of oxidized reovirus mRNA. In this report, we show that IF from poliovirus-infected cells contain significantly lower levels of CBPs as determined by the crosslinking assay. In addition, we demonstrate that IF preparations from infected cells have the ability to effect this reduction and can also impair the restoring activity of IF from mock-infected cells.

## MATERIALS AND METHODS

**Cells and Viruses.** Mouse L-929 cells and HeLa S3 cells were grown in suspension in 10% calf serum. Infection of L cells with reovirus Dearing 3 strain (10 plaque-forming units/cell) and virus purification were carried out as described (22). Infection of HeLa cells with poliovirus Mahoney 1 strain (10-20 plaque-forming units/cell) and virus isolation were according to Rose *et al.* (9). Sindbis virus infection of chicken embryo fibroblasts (23) and encephalomyocarditis virus (EMC) infection of L cells (24) were as described.

**Preparation of RNAs.** Synthesis of [ $^3H$ ]methyl-labeled reovirus mRNA to a specific activity of  $\approx 80,000$  cpm/ $\mu g$  with viral cores in the presence of S-adenosylmethionine (specific activity

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Abbreviations: kDal, kilodalton(s); CBP, cap-binding protein; 24-CBP, 24-kDal CBP; IF, initiation factors; VSV, vesicular stomatitis virus; EMC, encephalomyocarditis virus; STNV, satellite tobacco necrosis virus;  $m^7GDP$ , 7-methylguanosine diphosphate; PhMeSO<sub>2</sub>F, phenylmethylsulfonyl fluoride.

$\approx 70$  Ci/mmol, New England Nuclear; 1 Ci =  $3.7 \times 10^{10}$  becquerels) and periodate oxidation were according to Muthukrishnan *et al.* (25). EMC virus RNA was isolated as described (24). Total Sindbis RNA was obtained from infected chicken embryo fibroblasts and consisted of approximately 85% 26S RNA, the remainder being the 42S RNA species (23).

**Preparation of Cell Extracts and IF.** Cell-free extracts from poliovirus-infected or mock-infected HeLa cells were prepared at 3 hr after infection, essentially as described before (9), except that the extracts were not preincubated under translation conditions, but were dialyzed for 2 hr against buffer containing 90 mM KOAc, 10 mM Hepes buffer at pH 7.6, 1.5 mM Mg(OAc)<sub>2</sub>, and 1 mM dithiothreitol before freezing. Initiation factors were prepared essentially as described (26). Briefly, S10 extracts were centrifuged at 48,000 rpm for 2 hr in a Beckman SW 50.1 rotor. The ribosomal pellet was resuspended in buffer containing 0.1 M KCl, 20 mM Hepes at pH 7.5, 1 mM dithiothreitol, 0.2 mM EDTA, and 0.2 mM phenylmethylsulfonyl fluoride (PhMeSO<sub>2</sub>F), and 3 M KCl was added to a final concentration of 0.6 M. The mixture was stirred for 30 min and centrifuged as above, and the supernatant was dialyzed for 4 hr against 100 mM KOAc/20 mM Hepes, pH 7.5/1 mM dithiothreitol/0.2 mM EDTA/0.2 mM PhMeSO<sub>2</sub>F.

**Binding and Crosslinking of mRNA to IF.** [<sup>3</sup>H]Methyl-labeled oxidized reovirus mRNA was incubated with IF preparations under the conditions described in the figure legends for 10 min at 30°C in a final volume of 30  $\mu$ l. After incubation, 3  $\mu$ l of 0.2 M NaBH<sub>3</sub>CN was added and the mixture was left on ice for 3 hr, followed by the addition of 3  $\mu$ l of RNase A (5 mg/ml) and incubation for 30 min at 37°C to degrade the mRNA. The latter step was carried out according to the modification of Hansen and Ehrenfeld (27). Crosslinked proteins were resolved in NaDodSO<sub>4</sub>/12.5% polyacrylamide gels, followed by treatment with 2,5-diphenyloxazole/dimethyl sulfoxide or EN<sup>3</sup>HANCE (New England Nuclear) and exposure of Kodak X-Omat XR-1 film at -70°C as described (14).

**In Vitro Protein Synthesis.** Translation in HeLa cell extracts was carried out essentially according to Rose *et al.* (9). Incubation mixtures at a final volume of 25  $\mu$ l contained 13  $\mu$ l of micrococcal nuclease-treated S10 extract in 20 mM Hepes buffer, pH 7.6/130 mM KOAc/0.8 mM Mg(OAc)<sub>2</sub>/1 mM ATP/54  $\mu$ M GTP/9 mM creatine phosphate/0.6  $\mu$ g of creatine kinase/2 mM dithiothreitol/0.2 mM spermidine/11  $\mu$ M of each of 19 amino acids (minus methionine)/20  $\mu$ Ci of [<sup>35</sup>S]methionine (>1,000 Ci/mmol, New England Nuclear). After incubation for 1 hr at 37°C, 20- $\mu$ l samples were analyzed by electrophoresis on NaDodSO<sub>4</sub>/12.5% polyacrylamide gels, which were processed as described above.

## RESULTS

Cell-free extracts prepared from poliovirus-infected HeLa cells have a reduced ability to translate capped mRNAs, whereas translation of naturally uncapped mRNAs is not impaired (9, 11-13). Fig. 1 shows that our extracts had these characteristics. EMC RNA, which does not contain a cap structure at its 5' terminus (28), was translated with similar efficiencies in extracts from mock-infected and infected cells (Fig. 1, lanes 2 and 7, respectively), a finding that is consistent with earlier studies with *in vivo* and *in vitro* translation systems (29-31). In contrast, Sindbis mRNA (consisting of 26S and 42S RNA species—both capped mRNAs) was translated efficiently in extracts from mock-infected cells to yield mainly the coat protein ( $\approx 33$  kDal, lane 3), whereas translation was restricted in extracts from poliovirus-infected cells (compare lane 8 to lane 3). Crude IF preparations from mock-infected cells had very little effect on the translation of Sindbis mRNA in extracts from mock-infected

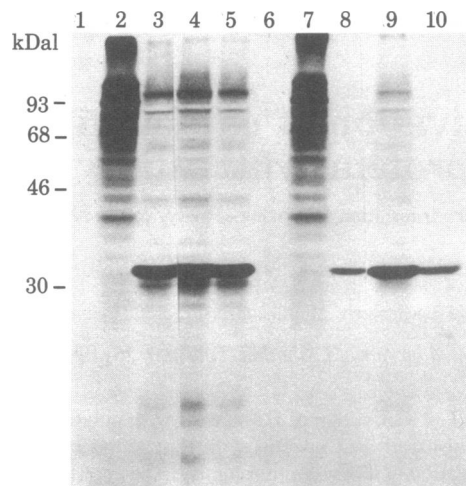
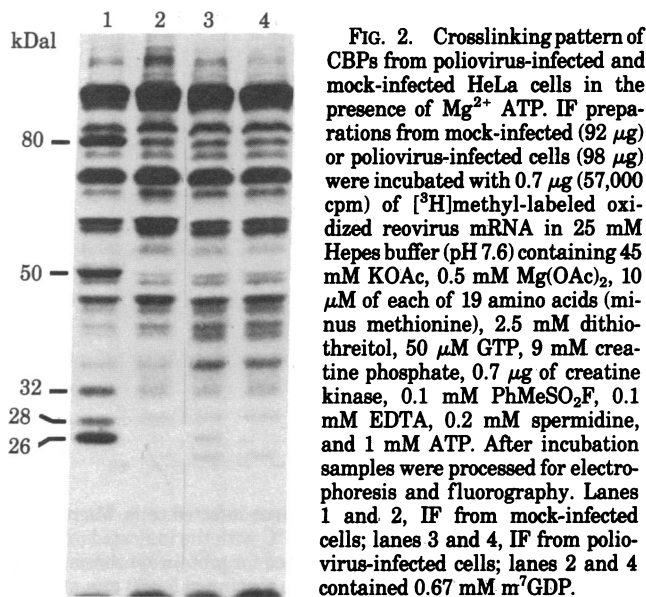


FIG. 1. Cell-free translation in poliovirus-infected and mock-infected HeLa cell extracts and the effect of IF. Translation was performed as described in the text and translation products were resolved on NaDodSO<sub>4</sub>/polyacrylamide gels and visualized by fluorography (14). Lanes 1-5, translation products in extracts from mock-infected HeLa cells. Lanes 6-10, translation products in extracts from poliovirus-infected HeLa cells. The following amounts of mRNA and IF were added: Lanes 1 and 6, no RNA; lanes 2 and 7, 0.5  $\mu$ g of EMC RNA; lanes 3 and 8,  $\approx 1$   $\mu$ g of Sindbis mRNA; lanes 4 and 9,  $\approx 1$   $\mu$ g of Sindbis mRNA plus 90  $\mu$ g of IF from mock-infected cells; lanes 5 and 10,  $\approx 1$   $\mu$ g of Sindbis mRNA plus 90  $\mu$ g of IF from poliovirus-infected cells.

cells (Fig. 1, compare lane 4 to lane 3), indicating that the IF do not contain any active component missing in the cell extracts. However, IF preparations from mock-infected cells stimulated the translation of Sindbis RNA in extracts from poliovirus-infected cells by approximately 4-fold (compare lane 9 to lane 8). In contrast, preparations from infected cells showed no such restoring activity: they had no effect on Sindbis mRNA translation in extracts of poliovirus-infected (Fig. 1, compare lane 10 to lane 8) or mock-infected cells (compare lane 5 to lane 3). These results confirm previous reports that a factor, crucial for translation of capped mRNAs and residing in high-salt wash of ribosomes, is inactivated in IF preparations from poliovirus-infected cells (8, 9, 11).

To test the hypothesis that the activity of a CBP is impaired in poliovirus-infected cells (9, 12, 13) we analyzed IF preparations by crosslinking to [<sup>3</sup>H]methyl-labeled oxidized reovirus mRNA. Crosslinking was performed in the presence of Mg<sup>2+</sup> ATP, which had previously been shown to be an absolute requirement for the cap-specific crosslinking of several polypeptides, other than the 24-CBP (19). The polypeptides from rabbit reticulocyte IF that required Mg<sup>2+</sup> ATP to crosslink had molecular masses of 28, 50, and 80 kDal and are referred to as CBPs throughout the text (17-19). Fig. 2 shows that oxidized reovirus mRNA could be crosslinked to several polypeptides in crude IF preparations from mock-infected HeLa cells (lane 1). Addition of m<sup>7</sup>GDP prevented the crosslinking of the 24-CBP\* in addition to the 28-, 50-, and 80-kDal polypeptides (Fig. 2, lane 2). Crosslinking of an  $\approx 32$ -kDal polypeptide was also inhibited by the addition of m<sup>7</sup>GDP, although crosslinking of this polypeptide has not been observed in rabbit reticulocyte IF. In-

\* Note that the CBP with the fastest mobility has been assigned a molecular mass of 26 kDal. This was also observed by Hansen and Ehrenfeld (27) and with IF from other species (unpublished results). However, because this protein was originally termed the 24-CBP (13, 14) we refer to it throughout the text as the 24-CBP or 24-kDal polypeptide in order to avoid confusion.



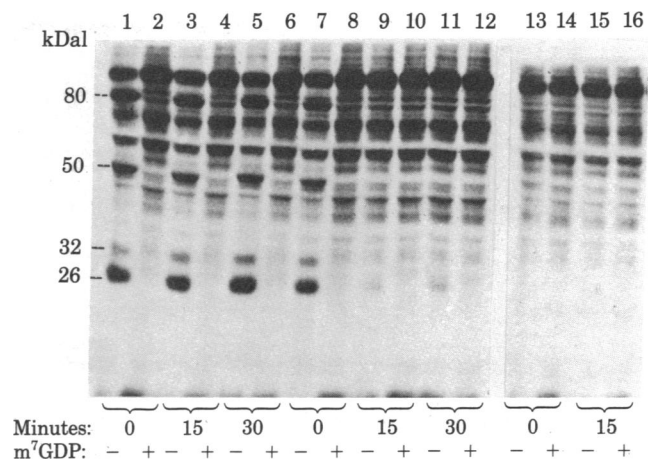
**FIG. 2.** Crosslinking pattern of CBPs from poliovirus-infected and mock-infected HeLa cells in the presence of  $Mg^{2+}$  ATP. IF preparations from mock-infected (92  $\mu$ g) or poliovirus-infected cells (98  $\mu$ g) were incubated with 0.7  $\mu$ g (57,000 cpm) of [ $^3$ H]methyl-labeled oxidized reovirus mRNA in 25 mM Hepes buffer (pH 7.6) containing 45 mM KOAc, 0.5 mM  $Mg(OAc)_2$ , 10  $\mu$ M of each of 19 amino acids (minus methionine), 2.5 mM dithiothreitol, 50  $\mu$ M GTP, 9 mM creatine phosphate, 0.7  $\mu$ g of creatine kinase, 0.1 mM  $PhMeSO_2F$ , 0.1 mM EDTA, 0.2 mM spermidine, and 1 mM ATP. After incubation samples were processed for electrophoresis and fluorography. Lanes 1 and 2, IF from mock-infected cells; lanes 3 and 4, IF from poliovirus-infected cells; lanes 2 and 4 contained 0.67 mM  $m^7GDP$ .

cubation of crude IF from poliovirus-infected cells with oxidized reovirus mRNA resulted in a markedly reduced level of crosslinking of the 24-, 28-, 32-, 50-, and 80-kDa polypeptides (Fig. 2, lane 3). Crosslinking of the 28-, 32-, 50-, and 80-kDa polypeptides was reduced to the level observed with IF from mock-infected cells in the presence of  $m^7GDP$  (compare lane 3 to lane 2). The low residual level of crosslinking of these proteins observed in IF from infected cells is also resistant to the addition of  $m^7GDP$  (compare lane 4 to lane 3), indicating that this is not cap specific. In contrast, it is noteworthy that the residual crosslinking of the 24-CBP observed in IF from poliovirus-infected cells is totally prevented by the addition of  $m^7GDP$  (lane 4).

Crude IF preparations from poliovirus-infected cells contain additional proteins, absent from mock-infected preparations, which can crosslink nonspecifically to oxidized reovirus mRNA. The most notable of these migrates slightly faster than the 24-CBP (lanes 3 and 4) and is probably the poliovirus capsid protein VP3, which is known to be a "sticky" protein and has been found in association with ribosomes from infected cells (32). We have observed this protein very consistently and believe that it serves as a useful and reliable marker of infection.

Crosslinking was also performed with  $(NH_4)_2SO_4$ -fractionated IF in the presence of  $Mg^{2+}$  ATP. The level of detectable CBPs was again markedly reduced in preparations from infected cells (data not shown). Most of the 24-CBP fractionated in the 0–40% saturated  $(NH_4)_2SO_4$  cut, consistent with previous findings that the 24-CBP copurifies with initiation factors eIF-3 and eIF-4B (12, 14, 15), both of which fractionate in the 0–40%  $(NH_4)_2SO_4$  cut of IF preparations (33). More recently, Hansen and Ehrenfeld have demonstrated that the 24-CBP is present in the 0–40%  $(NH_4)_2SO_4$  cut of IF from HeLa cells (27).

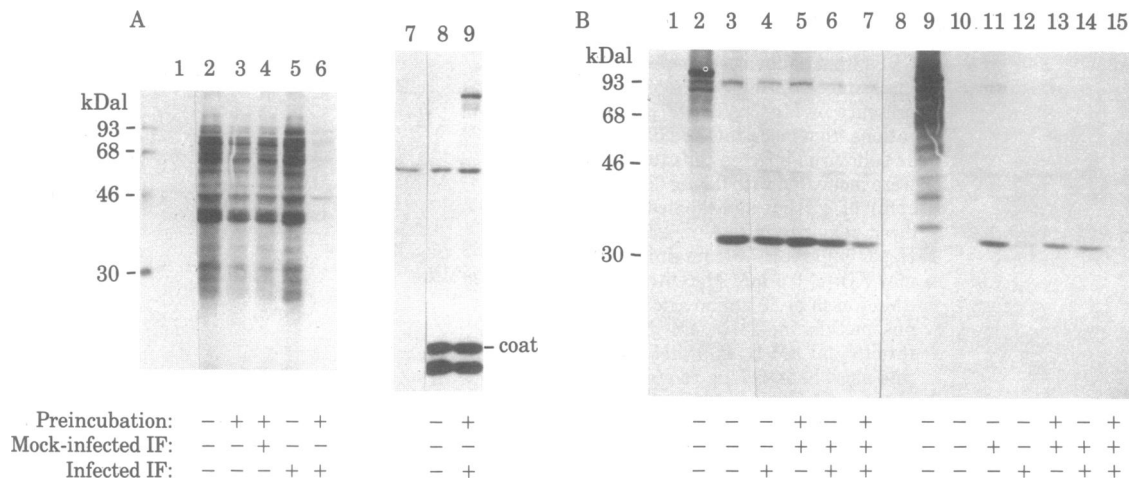
Rose *et al.* (9) have reported that cell-free extracts from poliovirus-infected cells contain an activity that slowly reduces the ability of uninfected extracts to translate capped mRNAs *in vitro*. It was of interest, therefore, to determine whether IF preparations from infected cells had such an activity and also whether these IFs could impair the crosslinking ability of the different CBPs. To this end, we preincubated IF from mock-infected cells with IF from poliovirus-infected cells prior to the crosslinking assay. Incubation of IF from mock-infected HeLa cells with [ $^3$ H]methyl-labeled oxidized reovirus mRNA resulted in crosslinking of several proteins (Fig. 3, lane 1). Addition of  $m^7GDP$  to the incubation mixture decreased the crosslinking



**FIG. 3.** Effect of mixing IF from mock-infected and poliovirus-infected cells on crosslinking of CBPs to mRNA. IF from mock-infected cells (92  $\mu$ g) were preincubated with IF from poliovirus-infected cells (36  $\mu$ g) at 37°C for the times indicated in the figure, prior to the addition of [ $^3$ H]methyl-labeled oxidized reovirus mRNA (0.5  $\mu$ g,  $\approx$ 42,000 cpm) under crosslinking conditions in the presence of 1 mM ATP as described in the legend to Fig. 2. As control experiments, IF from mock-infected and poliovirus-infected cells were preincubated separately. Each incubation was performed in the absence or presence of 0.67 mM  $m^7GDP$ . After incubation samples were processed for electrophoresis. Lanes 1–6, preincubation of IF from mock-infected cells; lanes 7–12, preincubation of IF from mock-infected cells with IF from poliovirus-infected cells; lanes 13–16, preincubation of IF from poliovirus-infected cells.

of the previously described 24-, 32-, 50-, and 80-kDa CBPs (lane 2; note that the 24- and 28-kDa polypeptides were not resolved in this experiment). Preincubation of IF from mock-infected cells for 15 min (lane 3) or 30 min (lane 5) did not impair their specific crosslinking ability. Simple mixing of IF from poliovirus-infected cells with IF from mock-infected cells did not diminish the crosslinking ability of the various CBPs in the latter fraction (lane 7). However, preincubation of this mixture for 15 min (lane 9) drastically diminished the ability of the CBPs to crosslink to mRNA. Nonspecific crosslinking of polypeptides, for example the 92-kDa protein, was not affected even after 30-min preincubation (lane 11). Again, it is apparent that there is residual crosslinking of the 50- and 80-kDa polypeptides that is no longer inhibited by  $m^7GDP$  (compare lanes 9 and 10), indicating that the residual level is probably due to nonspecific crosslinking of polypeptides with molecular weights similar to those of CBPs (see also Fig. 2). In contrast, after preincubation a fraction of the 24-CBP could still crosslink to mRNA, and this crosslinking was sensitive to  $m^7GDP$  (compare lane 10 to lane 9). This residual amount of 24-CBP was not abolished even after 30-min preincubation with IF from poliovirus-infected cells (lane 11). Lane 13 represents the crosslinking pattern of IF from poliovirus-infected cells that do not contain cap-specific crosslinkable proteins. Preincubation of this preparation had no effect on the crosslinking pattern (lane 15). These results indicate that IF from poliovirus-infected cells contain an activity that rapidly impairs the ability of the various CBPs to recognize the cap structure of the mRNA and would presumably effect a reduction in cellular protein synthesis. These results also exclude the possibility that IF from poliovirus-infected cells contain a preformed inhibitor of CBP function, because no effect could be observed without preincubation.

An important question to address was whether IF preparations from poliovirus-infected cells could also mediate the reduction in the ability of extracts from mock-infected cells to



**FIG. 4.** Effect on protein synthesis of preincubation of HeLa cell extracts with IF from mock-infected or poliovirus-infected cells. Micrococcal nuclease-treated HeLa cell extracts prepared from poliovirus-infected or mock-infected cells were preincubated at 37°C with the indicated amounts of IF from poliovirus-infected or mock-infected cells in 25- $\mu$ l incubation mixtures containing the components required for protein synthesis except for [ $^{35}$ S]methionine and mRNA. At the times indicated, mRNA and [ $^{35}$ S]methionine were added and incubation was continued for 60 min at 37°C. (A) Translation in mock-infected HeLa cell extracts with no added RNA (lanes 1 and 7), 2  $\mu$ g of reovirus mRNA (lanes 2–6), or 1  $\mu$ g of STNV RNA (lanes 8 and 9). IFs (20  $\mu$ g) were added where indicated in the figure and preincubation time was: lane 3, 25 min; lane 4, 25 min; lane 6, 12 min; and lane 9, 20 min. The autoradiograph for STNV translation products was exposed for a longer period than the one for reovirus translation products. (B) Translation in extracts of mock-infected cells (lanes 1–7) or poliovirus-infected cells (lanes 8–15) was programmed by 0.5  $\mu$ g of EMC RNA (lanes 2 and 9) or  $\approx$ 1  $\mu$ g of Sindbis RNA (lanes 3–7 and 10–15). No RNA was added in lanes 1 and 8. IFs (90  $\mu$ g) from mock-infected or poliovirus-infected cells were included as indicated and preincubation time was 20 min.

translate capped mRNAs that is observed after preincubation with extracts from poliovirus-infected cells (9). Fig. 4A is an autoradiograph of the translation products encoded by reovirus and satellite tobacco necrosis virus (STNV) mRNAs in extracts from mock-infected HeLa cells. Lanes 1 and 7 represent the endogenous translation products in the nuclease-treated extracts. Translation of reovirus mRNA yielded the various reovirus structural polypeptides (lane 2). Preincubation of the extract in the absence or presence of IF from mock-infected cells (lanes 3 and 4, respectively) slightly reduced the extent of translation, and addition of IF from infected cells without preincubation had no effect on translation of reovirus mRNA (lane 5). However, preincubation of the same mixture for 12 min dramatically curtailed translation (lane 6). STNV RNA was translated in mock-infected extracts to yield the 22-kDal coat protein and an 18-kDal prematurely terminated translation product (34) (Fig. 4A, lane 8), and could also be translated in poliovirus-infected extracts (data not shown). In contrast to the distinct inactivation of reovirus mRNA translation there was no detectable inhibition of STNV translation in extracts from mock-infected cells that were preincubated with IF from poliovirus-infected cells (compare lane 9 to lane 8). An unexplained synthesis of a 94-kDal polypeptide was observed in the preincubated cell extract (lane 9).

In a second set of experiments we attempted to determine whether IF from poliovirus-infected cells could also reduce the restoring activity of IF from mock-infected cells. In the data shown, EMC RNA was translated with higher efficiency in infected than in mock-infected cell extracts (Fig. 4B, lanes 9 and 2, respectively). Sindbis mRNA was translated efficiently in extracts from mock-infected cells to yield the coat protein and the 93-kDal B<sub>1</sub> precursor polypeptide (31) (lane 3), while 1/10th as much translation (as determined by densitometry tracing of the coat protein band) was observed in poliovirus-infected cell extracts (lane 10). Addition of IF from mock-infected cells with 30-min preincubation had no effect on translation of Sindbis mRNA (lane 5), whereas addition of IF from infected cells or a mixture of IF from mock-infected and infected cells

without preincubation slightly reduced translation (lanes 4 and 6). However, addition of the mixture to extracts from mock-infected cells followed by a 20-min preincubation resulted in approximately 60% inhibition of translation of Sindbis mRNA (compare lane 7 to lane 3). Thus, the slight inhibition observed in lanes 4 and 6 could be explained by the inhibitory effects of the infected IF during the translation incubation. In the translation system from poliovirus-infected cells, IF from mock-infected cells restored the ability to translate Sindbis mRNA (lane 11) but preincubation of these extracts with IF from mock-infected cells partially reduced the restoring activity by  $\approx$ 30% (lane 13). In contrast, IF from poliovirus-infected cells did not exhibit significant restoring activity (lane 12), and addition of IF from poliovirus-infected cells to the infected cell extract supplemented with IF from mock-infected cells without preincubation only partially inhibited the translation (40% inhibition, lane 14). This partial inhibition could reasonably be explained by inactivation of the restoring activity during the translation incubation. However, preincubation of extracts supplemented with IF from mock-infected cells with IF from infected cells resulted in a complete loss of the restoring activity of the mock-infected IF (lane 15). This result indicates that the IF from poliovirus-infected cells contain an activity that neutralizes the restoring activity. This activity is probably related to the activity that impairs the crosslinking ability of CBPs, thus lending support to the belief that the restoring activity resides in the CBPs.

## DISCUSSION

It has been suggested that the 24-CBP is inactivated during infection by poliovirus and that this inactivation mediates the shut-off of host protein synthesis. More recently it has been shown that other polypeptides (28, 50, and 80 kDal) can specifically recognize the cap structure (17, 19), and that higher molecular weight polypeptides purified by m<sup>7</sup>GDP affinity chromatography are essentially required for restoring translation of capped mRNAs in poliovirus-infected cell extracts (16). The 50- and 80-kDal polypeptides may correspond to eIF-4A

and eIF-4B, respectively, on the basis of the crosslinking characteristics of these factors (35).

In this study, we attempted to determine whether a change in the 24-CBP, the other CBPs, or both accompanies the shut-off of host protein synthesis exerted by poliovirus, by using the crosslinking assay (14). Our finding that all of the polypeptides capable of recognizing the cap structure lose their binding activity during poliovirus infection is consistent with what would be expected if the various CBPs in HeLa cells are structurally related, as is the case with CBPs of rabbit reticulocytes (18). In addition, the apparently coordinate inactivation of all the CBPs suggests that they are functionally related.

The reduction in the amount of detectable CBPs during poliovirus infection correlates both with the inability of extracts from infected cells to translate capped mRNAs and with the absence of restoring activity from IF of infected cells. This strongly suggests that functional CBPs are a vital component of the restoring activity. This suggestion is supported by data demonstrating the existence of activity that can, upon preincubation, impair both the restoring activity of IF from mock-infected cells and the ability of CBPs to recognize and crosslink to the cap structure. This activity resides in IF preparations from infected cells and rapidly impairs the ability of CBPs to crosslink to the cap structure. These findings are also consistent with the findings of Brown and Ehrenfeld (36), who demonstrated an activity in IF preparations from poliovirus-infected cells that specifically restricted the translation of capped mRNAs in reticulocyte lysate.

Our results appear to be at variance with those of Hansen and Ehrenfeld (27), who concluded that the cap-binding ability of 24-CBP as assayed by the crosslinking technique is not reduced during poliovirus infection. However, careful examination of their data reveals that the extent of crosslinking of the 24-CBP is distinctly lower in preparations from infected cells (figures 2 and 3 in ref. 27). In addition, we performed crosslinking analysis with  $(\text{NH}_4)_2\text{SO}_4$ -fractionated IF under similar conditions to these authors in the absence of ATP, and again we found substantially reduced amounts of 24-CBP in preparations from poliovirus-infected cells (data not shown).

The mechanism by which CBPs are inactivated during poliovirus infection is not clear, nor is it known whether the inactivating factor(s) is virally coded or induced. It is possible that CBPs become modified or degraded in poliovirus-infected cells. It may be possible to differentiate between these different possibilities by using monoclonal antibodies directed against CBPs.

We are grateful to D. Guertin for valuable assistance in preparation of viral mRNAs, A. LaFiandra and A. J. Shatkin for EMC virus, V. Stollar for Sindbis virus, and J. M. Clark, Jr., for STNV RNA. We are also grateful to Drs. A. J. Shatkin and W. Mushynski for critical reading of the manuscript. This research was supported by Grants from the National Cancer Institute and the Medical Research Council of Canada. K.A.W.L. is a recipient of a predoctoral research fellowship from the Cancer Research Society (Montreal).

1. Bablanian, R. (1975) in *Progress in Medical Virology*, ed. Melnick, J. L. (Karger, Basel), Vol. 19, pp. 40–83.
2. Baltimore, D. (1969) in *The Biochemistry of Viruses*, ed. Levy, H. B. (Dekker, New York), pp. 101–176.
3. Lucas-Lenard, J. M. (1979) in *The Molecular Biology of Picornaviruses*, ed. Perez-Bercoff, R. (Plenum, New York), pp. 78–99.
4. Leibowitz, R. & Penman, S. (1971) *J. Virol.* **8**, 661–668.
5. Kaufman, Y., Goldstein, E. & Penman, S. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 1834–1838.
6. Fernandez-Munoz, R. & Darnell, J. E. (1976) *J. Virol.* **18**, 719–726.
7. Ehrenfeld, E. & Lund, H. (1977) *Virology* **80**, 297–308.
8. Helentjaris, T. & Ehrenfeld, E. (1978) *J. Virol.* **26**, 510–521.
9. Rose, J. K., Trachsel, H., Leong, K. & Baltimore, D. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 2732–2736.
10. Doyle, M. & Holland, J. J. (1972) *J. Virol.* **9**, 22–28.
11. Helentjaris, T., Ehrenfeld, E., Brown-Luedi, M. L. & Hershey, J. W. B. (1979) *J. Biol. Chem.* **254**, 10973–10978.
12. Trachsel, H., Sonenberg, N., Shatkin, A. J., Rose, J. K., Leong, K., Bergmann, J. E., Gordon, J. & Baltimore, D. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 770–774.
13. Sonenberg, N., Rupprecht, K. M., Hecht, S. M. & Shatkin, A. J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4345–4349.
14. Sonenberg, N., Morgan, M. A., Merrick, W. C. & Shatkin, A. J. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 4843–4847.
15. Bergmann, J. E., Trachsel, H., Sonenberg, N., Shatkin, A. J. & Lodish, H. F. (1979) *J. Biol. Chem.* **254**, 1440–1443.
16. Tahara, S., Morgan, M. A. & Shatkin, A. J. (1981) *J. Biol. Chem.* **256**, 7691–7694.
17. Sonenberg, N., Guertin, D., Cleveland, D. & Trachsel, H. (1981) *Cell* **27**, 563–572.
18. Sonenberg, N. & Trachsel, H. (1982) in *Current Topics in Cellular Regulation*, eds. Horecker, B. L. & Stadtman, E. R. (Academic, New York), Vol. 21, pp. 65–88.
19. Sonenberg, N. (1981) *Nucleic Acids Res.* **9**, 1643–1656.
20. Hewlett, M. J., Rose, J. K. & Baltimore, D. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 327–330.
21. Nomoto, A., Lee, Y. F. & Wimmer, E. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 375–380.
22. Banerjee, A. K. & Shatkin, A. J. (1970) *J. Virol.* **6**, 1–11.
23. Cancedda, R. & Shatkin, A. J. (1979) *Eur. J. Biochem.* **94**, 41–50.
24. Eggen, K. L. & Shatkin, A. J. (1972) *J. Virol.* **9**, 636–645.
25. Muthukrishnan, S., Morgan, M., Banerjee, A. K. & Shatkin, A. J. (1976) *Biochemistry* **15**, 5761–5768.
26. Schreier, M. H. & Staehelin, T. (1973) *J. Mol. Biol.* **73**, 329–349.
27. Hansen, J. & Ehrenfeld, E. (1981) *J. Virol.* **38**, 438–445.
28. Frisby, D., Eaton, M. & Fellner, P. (1976) *Nucleic Acids Res.* **3**, 2771–2787.
29. Jen, G., Detjen, B. M. & Thach, R. E. (1980) *J. Virol.* **35**, 150–156.
30. Detjen, B. M., Jen, G. & Thach, R. E. (1981) *J. Virol.* **38**, 777–781.
31. Bonatti, S., Sonenberg, N., Shatkin, A. J. & Cancedda, R. (1980) *J. Biol. Chem.* **255**, 11473–11477.
32. Wright, P. J. & Cooper, P. D. (1974) *Virology* **59**, 1–20.
33. Schreier, M. H., Erni, B. & Staehelin, T. (1977) *J. Mol. Biol.* **116**, 727–753.
34. Salvato, M. S. & Fraenkel-Conrat, H. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 2288–2292.
35. Grifo, J. A., Tahara, S. M., Leis, J. P., Morgan, M. A., Shatkin, A. J. & Merrick, W. C. (1982) *J. Biol. Chem.* **257**, in press.
36. Brown, B. A. & Ehrenfeld, E. (1980) *Virology* **103**, 327–339.