The Cellular 68,000-\textit{M}_r Protein Kinase Is Highly Autophosphorylated and Activated Yet Significantly Degraded during Poliovirus Infection: Implications for Translational Regulation

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We investigated the possible translational regulatory roles played by the interferon-induced, double-stranded-RNA-activated protein kinase (P68) and its natural substrate, eucaryotic initiation factor 2 (eIF-2), in poliovirus-infected cells. We demonstrated that protein kinase P68 was both highly autophosphorylated and activated during poliovirus infection. In accordance with these results, immunoprecipitation analysis revealed that phosphorylation of the endogenous eIF-2 alpha subunit also increased in poliovirus-infected cells. We found that double-stranded RNA synthesized during infection likely induced the high levels of P68 autophosphorylation. To determine whether the increase in kinase activity also could be attributed to induction of P68 synthesis, physical levels of protein kinase were measured. It was unexpectedly found that P68 protein levels did not increase but rather dramatically declined in poliovirus-infected cells. Pulse-chase experiments confirmed that the protein kinase was significantly degraded during virus infection. We corroborated our in vivo observations by developing an in vitro assay for P68 degradation using cell extracts. The possible consequences of P68 degradation and increased eIF-2 alpha phosphorylation for protein synthesis regulation in poliovirus-infected cells are discussed.

Early after poliovirus infection, there is a selective and dramatic shutoff of host cell protein synthesis, whereas late after infection there is a decline in viral protein synthesis as well (6, 15, 31, 44). The mechanisms underlying these translational events have been intensely studied but still are not completely understood (for reviews, see references 28 and 51). The host shutoff is exerted primarily at the level of initiation and was found to correlate with degradation of a 220,000-dalton protein (P220), a component of eucaryotic initiation factor 4F (eIF-4F) (11). eIF-4F, also known as the cap-binding protein complex (CBPII), facilitates the binding of capped mRNAs to 43S initiation complexes (49). Thus, destruction of P220 would decrease the translatability of the capped host messages and favor the selective translation of uncapped poliovirus mRNAs (9). It has been found that sequences within the 5' noncoding region of poliovirus mRNAs contribute to their cap-independent translation (39). In two recent studies, it has been shown that translational initiation on poliovirus mRNA and the mRNA encoded by another picornavirus, encephalomyocarditis virus, likely occurs by binding of ribosomes to an internal 5' noncoding sequence (22, 40).

There is accumulating evidence that poliovirus protease 2A is responsible for P220 cleavage. In cells infected by a poliovirus mutant defective in functional 2A, P220 was not cleaved, and as a result, host cell protein synthesis was not selectively inhibited (3). A recent study has definitively shown that 2A, through the action of an unidentified cellular factor, was responsible for the degradation of P220 (29). Bonneau and Sonenberg have demonstrated, however, that P220 cleavage was not sufficient for the total shutoff of cellular protein synthesis (5). In cells infected by poliovirus in the presence of guanidine (an inhibitor of viral replication), P220 cleavage was complete but host translational inhibition was not. On the basis of these data, it was suggested that additional events were necessary to ensure total host shutoff during poliovirus infection. These mechanisms, as well as those responsible for the decline of viral protein late after infection, are unknown.

In this study, we examined the possible translational regulatory roles played by the interferon-induced protein kinase (referred to as P68 on the basis of its Mr of 68,000 but also referred to as DAI and PI/eIF-2 kinase; 13, 16–19, 32, 46) and its natural substrate, eIF-2, in poliovirus-infected cells. We were particularly interested in examining the phosphorylation states of these two proteins because of recent studies that showed that a protein kinase identified as the double-stranded-RNA (dsRNA)-activated protein kinase P68 was activated during poliovirus infection but, because of the presence of a specific inhibitor, did not result in increased phosphorylation of the eIF-2 alpha subunit (41, 42). We present evidence that the 68,000-M_r protein kinase is both highly autophosphorylated and activated during poliovirus infection. However, in contrast to the earlier studies (41, 42), we demonstrated that the increased kinase activity resulted in an increase in the endogenous levels of eIF-2 alpha phosphorylation. Unexpectedly, we found that despite its increased activity, the P68 kinase, like P220, was significantly degraded during poliovirus infection. These events and their possible effects on the regulation of protein synthesis in poliovirus-infected cells are discussed.

MATERIALS AND METHODS

Cells and virus. HeLa cells were grown in monolayer in Dulbecco modified Eagle medium containing 10% calf serum. Suspension HeLa cells were grown in Joklik modified minimum essential medium supplemented with 5% calf serum. Poliovirus type 1 (Mahoney strain), a generous gift
from Nahum Sonenberg and Jerry Pelletier, was grown in suspension HeLa cells. The polivirus was titrated by plaque assay on HeLa cells. HeLa cells were infected at a multiplicity of infection of 20 for the experiments reported in Results.

**Labeling conditions and immunoprecipitation analysis.** HeLa cells were labeled with either (i) $^{35}$S]methionine (500 μCi/ml) in minimum essential medium lacking methionine or (ii) $^{32}$P, (250 μCi/ml) in Dulbecco modified Eagle medium lacking phosphate for the times indicated in Results. After being labeled, the cells were washed with ice-cold Hanks balanced salt solution and then disrupted in lysis buffer (10 mM Tris hydrochloride [pH 7.5], 50 mM KCl, 1 mM dithiothreitol, 2 mM MgCl₂, 0.2 mM phenylmethylsulfonyl fluoride, 1,000 U of aprotinin per ml, 1% Triton X-100). The labeled extracts were centrifuged, and the supernatant was either boiled in 2× electrophoresis disruption buffer for direct analysis of labeled proteins or subjected to immunoprecipitation analysis.

For immunoprecipitations, extracts were diluted in buffer I (20 mM Tris hydrochloride [pH 7.5], 50 mM KCl, 400 mM NaCl, 1 mM EDTA, 100 U of aprotinin per ml, 1 mM dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride, 20% glycerol, 1% Triton X-100). The extracts were reacted for 2 h at 4°C with either a monoclonal antibody (MAB) to P68 (30) or a polyclonal antibody to eIF-2 or tubulin. The three subunits of eIF-2 (alpha, beta, and gamma) are efficiently immunoprecipitated with the antibody to purified eIF-2 (25). The polyclonal antibody to tubulin was a generous gift from Sue Moyer. For the eIF-2 and tubulin immunoprecipitations, protein A-agarose was added and the mixture was incubated for one additional hour at 4°C. The precipitates were then washed four times with buffer I and three times with buffer II (10 mM Tris hydrochloride [pH 7.5], 100 mM KCl, 0.1 mM EDTA, 100 U of aprotinin per ml, 20% glycerol). The washed immunoprecipitates were boiled in 2× electrophoresis disruption buffer and analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE).

**In vitro P68 kinase reaction.** The P68 protein kinase analyzed for functional activity was first purified from cell extracts on MAb-Sepharose as described above. Following the washings in buffer II, the immunoprecipitates were suspended in buffer II containing 2 mM MgCl₂, 1 mM MnCl₂, 5 μM [γ-$^{32}$P]ATP (50 to 100 Ci/mmol), and 0.5 μg of purified eIF-2B (Boal and B. Safer, manuscript in preparation). The kinase reaction was allowed to proceed at 30°C for 30 min, after which the reaction was stopped by addition of 2× electrophoresis disruption buffer and boiling. The samples were then analyzed by SDS-PAGE.

**RNA isolation and activation of P68 in vitro.** Suspension HeLa cells were infected with polivirus in minimum essential medium containing 2% calf serum at a multiplicity of infection of 20. [3H]uridine was included in the medium to facilitate detection of RNA in the subsequent column purification steps. At 4.5 h postinfection, the cells were pelleted and washed three times with phosphate-buffered saline. Total RNA was isolated from cells as described previously (23). The RNA was quantitated and either subjected to column chromatography or analyzed for its ability to activate P68 in vitro. Total RNA from polivirus-infected cells was separated into soluble, ribosomal, and dsRNA fractions by column chromatography on a Whatman cellulose column (20 by 1.5 cm) as described previously (14). The column elution steps used to obtain soluble, ribosomal, and dsRNAs, respectively, were as follows: 65% STE (0.1 M NaCl, 0.001 M EDTA, 0.05 M Tris [pH 6.8]-35% ethanol, 85% STE-15% ethanol, and 100% STE. The RNA from each elution step was pooled and analyzed for its ability to activate the P68 protein kinase present in the ribosomal salt wash (RSW) prepared from interferon-treated Daudi cells as previously described (24). Different concentrations of column-purified or total RNA were added to the RSW in a reaction containing buffer III (20 mM HEPES [N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid; pH 7.5], 50 mM KCl, 2 mM MgCl₂, 2 mM MnCl₂, 5 mM mercaptoethanol, 5μM [γ-$^{32}$P]ATP (50 to 100 Ci/mmol)). The reactions were incubated for 30 min at 30°C and then stopped by addition of 2× electrophoresis disruption buffer and boiling. The samples were then subjected to SDS-PAGE.

**In vitro assay for P68 degradation.** HeLa cell extracts from mock infected cells or cells infected with polivirus for 3.5 h were prepared in lysis buffer without protease inhibitors. As a source of radiolabeled P68, extracts were prepared as described above from HeLa cells labeled with [35]Smethionine and treated with human lymphoblastoid interferon for 9 h. The [35]Smethionine-labeled extract was mixed with either the mock- or polivirus-infected extract for the times indicated in Results. Following mixing, radiolabeled P68 was immunopurified from the mixture with P68 MAB-Sepharose and subjected to SDS-PAGE as described above.

**RESULTS**

**Kinetics of protein synthesis during polivirus infection of HeLa cells.** We analyzed the pattern of protein synthesis during polivirus infection by labeling infected cells with [35]Smethionine every 30 min for 5 h. Samples were removed, and the trichloroacetic acid-precipitable counts per minute were determined; the results are shown in Fig. 1. There was a dramatic overall decrease in mRNA translation at 2 h postinfection, likely because of P220 cleavage, followed by partial recovery at 3 h. SDS-PAGE analysis revealed that only viral proteins were synthesized at 3 h postinfection (data not shown). Viral protein synthesis then declined significantly between 4 and 5 h postinfection, possibly as a result of eIF-2 alpha phosphorylation (see below). Since these results were in agreement with earlier reports (6, 44), we proceeded to our analysis of the P68
protein kinase and its possible role in translational regulation
in poliovirus-infected cells.

**P68 kinase is highly activated and autophosphorylated during poliovirus infection.**

It was previously reported that increased activity of a protein kinase is present in the RSW of poliovirus-infected cells (41). We wanted to determine whether this protein kinase was identical to the interferon-induced, dsRNA-activated kinase P68 (13, 16, 17, 19, 32, 46). P68 is characterized by two kinase activities, an autophosphorylation or activation reaction and a kinase activity on its exogenous substrates, such as the alpha subunit of eIF-2. The autophosphorylation of the cellular protein kinase during poliovirus infection was examined by using a MAAb specific for P68. Cells were either mock or poliovirus infected and labeled with $^{32}$P, from 1.5 to 3 and 3.5 to 5 h postinfection (Fig. 2A). The P68 kinase from infected cells was already approximately 10-fold more autophosphorylated than the P68 in mock-infected cells by 3 h postinfection and remained high at 5 h postinfection (compare lane A with lanes B and C). This is representative of several independent experiments in which the autophosphorylation in poliovirus-infected cells consistently increased 5- to 10-fold over that in mock-infected cells. To determine whether the increase in autophosphorylation of P68 correlated with an increase in activity, phosphorylation of purified eIF-2 by the activated P68 was measured. For this analysis, the protein kinase was immunopurified from poliovirus-infected cells at 3 and 5 h postinfection by using the MAAb and incubated with exogenously added eIF-2 in the presence of [γ$^{32}$P]ATP (Fig. 2B). The P68 activity in poliovirus-infected cells increased approximately fourfold by 3 h postinfection (lane B) compared with the P68 in mock-infected cells (lane A) and remained elevated as late as 5 h postinfection (lane C), as measured by enhanced phosphorylation of the eIF-2 alpha subunit (see the densitometer scan for quantitation [Fig. 2C]). Thus, the increase in autophosphorylation of P68 during poliovirus infection correlated with an increase in kinase activity.

**Activation of P68 results in an increase in phosphorylation of endogenous eIF-2 alpha during poliovirus infection.** It was critical to test whether this increased P68 activity resulted in enhanced levels of endogenous eIF-2 alpha phosphorylation, since profound effects on protein synthesis initiation have been reported to result from such increases in phosphorylation (7, 8, 24, 25, 37, 47, 48, 50, 52). Phosphorylated eIF-2 alpha traps eIF-2B, which is normally responsible for recycling eIF-2-GDP to eIF-2-GTP. The end result is a limitation in the amount of functional eIF-2 available for protein synthesis initiation (21, 27, 38, 45). Cells were infected and labeled with $^{32}$P, from 1.5 to 3 and 3.5 to 5 h after poliovirus infection, and mock-infected cells labeled from 1.5 to 3 h were used as a control. Immunoprecipitation of the labeled extracts was performed with antibody to purified eIF-2 (26). The phosphorylated levels of eIF-2 alpha increased in poliovirus-infected cells by 3 h and remained elevated at 5 h postinfection (Fig. 3a, compare lane A with lanes B and C).
Identification of the eIF-2 alpha band was verified by allowing labeled extracts to compete with an excess of cold eIF-2 during the immunoprecipitation procedure (data not shown). As measured by densitometric scanning of radiolabeled bands (Fig. 3b), eIF-2 alpha phosphorylation was found to increase approximately threefold over that in mock-infected cells at 3 and 5 h postinfection. In some experiments, the increases were only twofold, but most importantly, we consistently observed an increase in alpha phosphorylation in several independent experiments. To rule out that these results were due to an in vitro phosphorylation reaction occurring after cell disruption, cells were lysed in the presence of 2 mM EDTA (in the absence of MgCl₂) and 50 mM NaF. Under these conditions, there should be minimal phosphorylation or dephosphorylation in vitro. The results were comparable to those shown in Fig. 3a, indicating that phosphorylation of the eIF-2 alpha subunit did occur in vivo (data not shown). Therefore, the increase in P68 auto-phosphorylation and activity resulted in an increase in the phosphorylation of endogenous eIF-2 alpha during poliovirus infection.

Poliovirus dsRNA induces autophosphorylation of P68 during poliovirus infection. We next wanted to determine what caused the increase in P68 autophosphorylation and activity during poliovirus infection. A likely candidate was the dsRNA synthesized in poliovirus-infected cells (1, 2, 4), particularly since the kinase is known to be activated by dsRNA (16, 17, 19, 35). Further, the dsRNA from poliovirus-infected cells previously was shown to inhibit both cellular and viral mRNA translation in vitro (6, 10). We first analyzed the activation of P68 in the RSW fraction of interferon-treated Daudi cells by total RNA prepared from mock- and poliovirus-infected cells (Fig. 4a). As a control, reovirus dsRNA (40 ng/ml), which is known to be an efficient activator of the kinase, was used (lane B). RNA from poliovirus-infected cells activated P68 to the greatest extent at a concentration of 10 μg/ml (approximately sixfold as measured by densitometric scanning of the radioactivity (lane G)), whereas at higher concentrations of RNA (100 μg/ml) activation was only twofold (lane H). The RNA (10 μg/ml) prepared from mock-infected cells activated the kinase to a much lesser extent (less than twofold; lane D). To examine which component of total RNA from poliovirus-infected cells was responsible for the activation of P68, the RNA was separated into soluble-RNA-, ribosomal-RNA-, and dsRNA-containing fractions on a Whatman cellulose column (14). Each fraction was analyzed subsequently for its ability to activate P68 in vitro. There was little or no activation of the P68 kinase by either the soluble-RNA- or ribosomal-RNA-containing fraction from poliovirus-infected cells (data not shown). In contrast, the dsRNA-containing fraction significantly activated P68 in the RSW at RNA concentrations of 1 and 10 μg/ml (Fig. 4b, lanes E and F). We therefore conclude that dsRNA synthesized during poliovirus infection is probably responsible, at least in part, for the enhanced P68 activation just described.

P68 protein kinase is dramatically degraded during poliovirus infection. To determine whether the increases in P68 activity in poliovirus-infected cells also could be attributed to induction in the physical levels of P68, steady-state levels of the kinase were measured by labeling the cells continuously with [35S]methionine from 0 to 3 h after mock or poliovirus infection. P68 was subsequently immunoprecipitated from the radiolabeled cell extracts (Fig. 5A). P68 from poliovirus-infected cells (lane B) was present in dramatically reduced amounts compared with that of mock-infected cells (lane A). Under these labeling conditions, we did not observe dramatic differences in the total pattern of cellular protein synthesis in mock- or poliovirus-infected cells (data not shown). However, to ensure that the decrease in P68 levels was not a consequence of host shutoff or P68 mRNA degradation, pulse-chase experiments were performed. Cells were labeled with [35S]methionine for 9 h before infection with poliovirus. The label was washed out, and cells were either mock or poliovirus infected in the presence of medium containing cold methionine. P68 was immunoprecipitated from cell extracts prepared at 0, 1.5, or 3 h postinfection (Fig. 5B). The protein kinase, as expected, was dramatically significantly degraded by 3 h postinfection (lane F), whereas P68 kinase was readily detected in mock-infected cells at the same time (lane C). We were unable to detect any P68-specific degradation products in poliovirus-infected cells. The small amount of P68 degradation in mock-infected cells is likely due to normal turnover of the protein, which reportedly has a t½ of 6 to 7 h (20). As a control, we immunoprecipitated tubulin from mock- and poliovirus-infected cells which, in contrast to P68, was stable for up to 3 h postinfection (Fig. 5C). We also found that the protein levels of the alpha, beta, and gamma subunits of eIF-2 remained constant throughout poliovirus infection (data not shown). We performed controls to exclude the possibility that the reduced detection of P68 in poliovirus-infected cells was due to lack of recognition by the MAb, possibly because of blocked reactive epitopes. (i) Extracts from mock- and poliovirus-infected cells were boiled for 1 min in the presence of 1% deoxycholate and 0.1% SDS before immunoprecipitation with the MAb. (ii) Immunoprecipitations were performed instead with a polyclonal antibody to the protein kinase. In each case, the results were identical to those
FIG. 5. P68 protein kinase is dramatically degraded during poliovirus infection. (A) P68 was immunoprecipitated from extracts prepared from cells continuously labeled with [35S]methionine from 0 to 3 h after mock (lane A) or poliovirus (lane B) infection. The positions and sizes (103) of molecular weight markers are shown to the left of panels A to C. (B) Pulse-chase analysis of P68 levels. Cells previously labeled for 9 h with [35S]methionine were infected with poliovirus in medium containing cold methionine. P68 was subsequently immunoprecipitated from cell extracts prepared at 0 (lane A), 1.5 (lane B), and 3 (lane C) h after mock infection or at 0 (lane D), 1.5 (lane E), and 3 (lane F) h after poliovirus infection. (C) Tubulin was immunoprecipitated from similar extracts prepared as for panel B. The lanes were identical to those of panel B. (D) Cells previously labeled for 9 h with [35S]methionine were infected with poliovirus in medium containing cold methionine. P68 was immunoprecipitated from whole-cell extracts prepared in lysis buffer containing 0.5% SDS, 0.5% sodium deoxycholate, and 1% Triton X-100 at 0 (lane A) and 3 (lane B) h after poliovirus infection.

Presented above (data not shown). Further, it was necessary to rule out the possibility that P68 was undetected because of its sequestration or reduced solubility, as reported for the c-myc protein during heat shock (12, 34). To ensure complete solubilization of P68, extracts from poliovirus-infected cells subjected to pulse-chase analysis were treated with 0.5% SDS–0.5% deoxycholate–1% Triton X-100 (34). The P68 levels were significantly lower (approximately 10-fold) in poliovirus-infected cells at 3 h postinfection (Fig. 5D, compare lanes A and B), confirming our previous analysis. It should be emphasized that P68, although significantly degraded, was still detectable at low levels in poliovirus-infected cells. These data, together with the results of the analysis of the phosphorylation state of P68, suggest that the remaining undegraded P68 protein is highly autophosphorylated during poliovirus infection, thus accounting for its increased activity and the resulting enhanced levels of eIF-2 alpha phosphorylation (Fig. 2 and 3).

We next developed an in vitro assay for P68 degradation both to corroborate our in vivo findings and to begin to define the mechanisms responsible for the degradation. Extracts from interferon-treated, [35S]methionine-labeled HeLa cells were mixed with either mock-infected extracts or extracts from cells infected with poliovirus for 3.5 h. The extracts were incubated for 15 min at 30°C (Fig. 6, lanes A and B) or at 0°C (lanes C and D), and P68 was immunoprecipitated. Significant degradation of P68 occurred when the labeled extracts were mixed at 30°C with poliovirus-infected extracts (lane B) but not when they were mixed with either mock-infected extracts (lane A) or poliovirus-infected extracts (lane B) and incubated for 15 min at 30°C. Subsequently, P68 was immunoprecipitated from the mixture. As a control, the labeled extracts were also incubated for 15 min at 0°C with 60 μl of mock (lane C) or poliovirus (lane D)-infected extract, followed by immunoprecipitation of the P68 protein kinase. Immunoprecipitates were analyzed on an SDS–10% polyacrylamide gel. The positions and sizes (103) of molecular weight markers are shown on the left.
with uninfected extracts (lane A). As expected, little degradation was detected, even by poliovirus extracts, with incubation at 0°C (lanes C and D). These results show that poliovirus-induced proteolysis of P68 also occurred efficiently in vitro.

**DISCUSSION**

We demonstrated that the P68 protein kinase was both highly autoprophorylated and activated during poliovirus infection. This increase in kinase activity was readily detected by 3 h postinfection (Fig. 2), even though P68 was significantly (but not totally) degraded by this time (Fig. 5). These seemingly paradoxical results can be explained if one assumes that the remaining undegraded protein kinase molecules are autoprophorylated (and thus activated) to high levels which persist until at least 5 h postinfection (Fig. 2).

We propose that poliovirus-specific dsRNAs synthesized during infection are responsible for this activation of the kinase (Fig. 4). This may not be unique to the poliovirus system, since it has similarly been suggested that dsRNA synthesized during mutant adenovirus infection may activate the P68 protein kinase (35).

As a result of enhanced kinase activity, the levels of eIF-2 alpha phosphorylation in poliovirus-infected cells also increased. It has been well documented in other systems that increased alpha phosphorylation has negative effects on protein synthesis initiation, since phosphorylation of eIF-2 prevents recycling of eIF-2-GDP to eIF-2-GTP by the recycling factor IF-2B because the latter is trapped in an inactive complex with eIF-2-GDP (21, 27, 33, 38, 45). For example, in heat-shocked, serum-deprived, and amino acid-starved cells, there is a direct correlation between increases in eIF-2 alpha phosphorylation and inhibition of protein synthesis (7, 8, 48). In addition, during infection by the virus-associated RNA I-negative adenovirus mutant d331, the kinase is highly activated and the resulting phosphorylation of eIF-2 alpha shuts down protein synthesis almost completely (24, 26, 37, 47, 50, 52). It is therefore reasonable to assume that the dramatic decrease in overall protein synthesis which occurred from 4 to 5 h postinfection (Fig. 1) results from enhanced P68 activity, eIF-2 alpha phosphorylation, and continued depletion of available initiation factors. Although we observed alpha phosphorylation at 3 h after poliovirus infection, functional eIF-2 and eIF-2B are probably not yet limiting at this time, since protein synthesis still occurs, albeit at lower levels than in uninfected cells (6; Fig. 1). Even though the shift from cellular to viral protein synthesis occurs at around 3 h in our system, we have no evidence suggesting that increased alpha phosphorylation plays a direct role in host shutoff.

As previously mentioned, Ransone and Dasgupta have reported that, despite activation of the protein kinase during poliovirus infection, there was no detectable increase in eIF-2 alpha phosphorylation (41). Further, they have begun characterization of the inhibitor of alpha phosphorylation present in poliovirus-infected cells (42). We cannot rule out the possibility that such an inhibitor functions in poliovirus-infected cells to prevent eIF-2 alpha phosphorylation from increasing above the levels we observed. However, there are alternative explanations for the discrepancy in our results. (i) Ransone and Dasgupta examined the endogenous phosphorylation of eIF-2 alpha which was present only in the RSW fraction of poliovirus-infected cells, whereas we measured the total cellular content of eIF-2 alpha. (ii) Our detection assay may be more sensitive, since we used monospecific antiserum to identify the relatively nonabundant cellular protein eIF-2 alpha, whereas Ransone and Dasgupta were forced to rely on twodimensional gel analysis without the benefit of an antibody. (iii) There is no mention of including NaF in the cell disruption buffers to protect against phosphatases which might act during cell extraction and RSW preparation.

In contrast to the poliovirus system, other viruses, including adenovirus, influenza virus, and vaccinia virus, encode mechanisms to block P68 activation (24, 26, 37, 43, 47, 50, 52). In the adenovirus system, the virus-encoded virus-associated RNA I complexes with the kinase to down regulate P68 activity (24), whereas recent experiments in our laboratory suggest that the influenza-virus-encoded inhibitor is not an RNA but rather a protein (Katze, unpublished data). Recent evidence suggests that, in addition to blocking kinase activation, vaccinia virus may induce P68 degradation (20). One could only hypothesize why P68 activation is not blocked in poliovirus-infected cells as it is during infection by these other viruses. It is possible that an activated kinase is needed to phosphorylate proteins necessary for virus replication. In support of this, Morrow et al. (36) have reported that the activated protein kinase may be essential for efficient poliovirus RNA replication.

It is tempting to speculate that, without significant proteolysis of P68, eIF-2 alpha phosphorylation levels would be even higher (resulting in total depletion of functional eIF-2) and eIF-2B is translocated 4 h postinfection, when viral proteins must be synthesized.

It is intriguing that poliovirus induces the degradation of two proteins, both of which have important roles in translational regulation: P68, the dsRNA-activated protein kinase, and P220, an essential component of the cap-binding protein complex. Efforts are in progress to determine whether similar mechanisms operate to cause the proteolysis of these two proteins.

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**LITERATURE CITED**


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