# Inhibitory activity for the interferon-induced protein kinase is associated with the reovirus serotype 1 σ3 protein

(eukaryotic protein synthesis initiation factor 2α/translational control/double-stranded RNA)

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**ABSTRACT** In this report we demonstrate that reovirus serotype 1-infected cells contain an inhibitor of the interferon-induced, double-stranded RNA (dsRNA)-dependent protein kinase. We provide evidence that suggests that the virus-encoded σ3 protein is likely responsible for this kinase inhibitory activity. We could not detect activation of the dsRNA-dependent protein kinase in extracts prepared from either interferon-treated or untreated reovirus serotype 1-infected mouse L cells under conditions that led to activation of the kinase in extracts prepared from either interferon-treated or untreated, uninfected cells. Extracts from reovirus-infected cells blocked activation of kinase in extracts from interferon-treated cells when the two were mixed prior to assay. The kinase inhibitory activity in extracts of reovirus-infected cells could be overcome by adding ≈100-fold excess of dsRNA over the amount required to activate kinase in extracts of uninfected cells. Kinase inhibitory activity in extracts of interferon-treated, virus-infected cells could be overcome with somewhat less dsRNA (≈10-fold excess). Most of the inhibitory activity in the extracts could be removed by adsorption with immobilized anti-reovirus σ3 serum or immobilized dsRNA, suggesting that the dsRNA-binding σ3 protein is necessary for kinase inhibitory activity. Purified σ3 protein, when added to reaction mixtures containing partially purified kinase, inhibited enzyme activation. Control of activation of this kinase, which can modify eukaryotic protein synthesis initiation factor 2, may be relevant to the sensitivity of reovirus replication to treatment of cells with interferon and to the shutoff of host protein synthesis in reovirus-infected cells.

Interferon (IFN) treatment of cells leads to the induction of a protein kinase (1) that may be involved in establishing the IFN-induced antiviral state (2, 3). This kinase requires interaction with ATP and a low concentration of double-stranded RNA (dsRNA) (0.01–1 μg/ml) to be activated (4). Active kinase phosphorylates the small (α) subunit of the eukaryotic protein synthesis initiation factor 2 (eIF-2) and a $M_r$ 67,000 polypeptide, designated $P_1$ (1, 5–7), that is thought to be a subunit of the kinase (8). Phosphorylation of eIF-2 on the α subunit leads to inactivation of this factor (9). Phosphorylation of $P_1$ correlates with activation of the enzyme (10).

Recently, several viruses that code for inhibitors of the IFN-induced, dsRNA-dependent protein kinase have been reported. The best characterized of the viral kinase inhibitors is the adenovirus VA I RNA (11). This small RNA binds to the protein kinase (12) and prevents activation of the kinase by dsRNA and ATP (11). The VA I RNA may be responsible for the resistance of adenovirus replication to pretreatment of cells with IFN, since a deletion mutant that lacks a VA I gene (*dl*331) is sensitive to the antiviral effects of IFN (13). In addition, cells infected with vaccinia virus contain an inhibitor of the kinase (14–16). This inhibitor is thought to be a protein that stoichiometrically interacts with dsRNA (17). The precise molecular nature and mode of action of the vaccinia virus kinase inhibitor have not been determined.

In addition to the viral inhibitors of the IFN-induced protein kinase, several groups have shown that histone proteins can inhibit this kinase (18, 19). We have recently found (32) that histones function as kinase inhibitors by binding to dsRNA and thus sequestering the dsRNA from the kinase. We, therefore, attempted to determine if another well-characterized dsRNA-binding protein, the reovirus σ3 protein (20), might possess inhibitory activity for the IFN-induced kinase. In this paper we show that reovirus serotype 1 (Lang)-infected mouse L cells contain an inhibitor of the dsRNA-dependent protein kinase and that the σ3 protein is necessary and may be sufficient for this kinase inhibitory activity. It therefore appears that inhibition of the IFN-induced protein kinase by a dsRNA-binding protein may be an alternative mechanism for escaping the effects of this putative antiviral protein kinase. We believe that this newly characterized activity of the reovirus σ3 protein may have relevance to the sensitivity of reovirus replication to pretreatment of cells with IFN and the shutoff of host protein synthesis during reovirus infection.

## MATERIALS AND METHODS

**Cells and Virus.** Mouse L cells were grown as monolayers, or in suspension, at 37°C in minimal essential medium supplemented with 5% fetal calf serum and gentamycin sulfate at 5 μg/ml. Reovirus serotype 1 (Lang) strain was obtained from Charles E. Samuel (University of California, Santa Barbara) and was plaque purified and propagated in mouse L cells.

**Preparation of Cell Extracts.** Suspension or monolayer cultures of mouse L cells were either infected with reovirus serotype 1 at a multiplicity of 10 plaque-forming units per cell, treated with partially purified mouse β-interferon (5.6 × 10⁷ units/mg, Lee Biomolecular Laboratories, San Diego, CA) at a concentration of 200 units/ml, infected with reovirus serotype 1 at 24 hr after IFN treatment, or left untreated. At 22 hr after infection, or 24 hr after IFN treatment, cells from suspension cultures were washed, swollen in hypotonic buffer, and disrupted by Dounce homogenization, as described (21). Alternatively, detergent extracts of monolayer cultures were prepared as described (22).

**Protein Purification.** σ3 was purified by dsRNA-Sepharose affinity chromatography, as described (20), except that commercially prepared poly(rI)·poly(rC)-Sepharose (Pharmacia) was used. The σ3 elution buffer was exchanged with kinase

reaction buffer lacking ATP and containing 0.5% Nonidet P-40 by centrifugation through a column of Sephadex G-25 (23).

dsRNA-dependent protein kinase was purified from the ribosomal fraction of IFN-treated mouse L cells (100 units of recombinant IFN-αA/D per ml, kindly provided by Michael Brunda, Hoffmann–La Roche) as described (24).

**Phosphorylation of Proteins *in Vitro*.** Reaction mixtures for *in vitro* phosphorylation of proteins contained 20 mM Hepes (pH 7.5), 90 mM KCl, 5 mM MgOAc, 1 mM dithiothreitol, 100 $\mu$M [$\gamma$-$^{32}$P]ATP (ICN; specific activity, 1 Ci/mmol; 1 Ci = 37 GBq), and extract from 3–10 × 10$^5$ cells, in a final volume of 25 $\mu$l. Reovirus dsRNA (25), or poly(rI)·poly(rC) (where indicated), was added at the concentrations indicated in each figure legend. Where indicated, bulk calf thymus histone proteins (Sigma) were added to the reaction mixtures at a concentration of 300 $\mu$g/ml, after preincubation of extract with dsRNA for 5 min on ice.

Phosphorylation of purified histone proteins by partially purified kinase was performed in the presence or absence of purified σ3 at a concentration of 2–5 $\mu$g/ml. Purified σ3 was preincubated with the indicated concentration of dsRNA on ice for 5 min, and then kinase, [$\gamma$-$^{32}$P]ATP, buffer components, and histone proteins were added.

All phosphorylation reactions were performed at 30°C for 15 min and quenched by addition of an equal volume of 2× NaDodSO$_4$/PAGE sample buffer (22). Samples were boiled for 2 min, and proteins were resolved by NaDodSO$_4$/PAGE through a 12% gel. Gels were stained with Coomassie R-250, dried, and subjected to autoradiography (22). Histone phosphorylation was quantitated by scanning densitometry of autoradiograms. For autoradiograms of reactions from crude extracts, peak areas were normalized to a band whose intensity did not change as a function of dsRNA.

**Antibody Adsorption.** Serum (kindly provided by P. W. K. Lee, Calgary, Alberta, Canada) from a rabbit that had been immunized with reovirus serotype 3 σ3 protein or serum from a nonimmunized animal was adsorbed to a 10-fold excess of a fixed preparation of *Staphylococcus aureus* (Boehringer Mannheim). After incubation for 1 hr at 4°C, the bacterial cells were extensively washed with isotonic buffer (20 mM Hepes/120 mM KCl/1 mM EDTA/1 mM benzamidine) and added to extracts prepared from reovirus-infected cells (the equivalent of 3 $\mu$l of serum was added to 10 $\mu$l of extract). After incubation for 1 hr at 4°C the bacterial cells were removed by centrifugation, and the supernatant solutions were used in protein phosphorylation reactions.

**dsRNA-Sepharose Adsorption.** Extracts were brought to 1 mM benzamidine, 100 units of heparin per ml, and 1 $\mu$M NAD$^+$ and added to washed poly(rI)·poly(rC)-Sepharose (Pharmacia), which had been preadsorbed with ovalbumin at a concentration of 10 mg/ml. The mixture was incubated at 4°C with occasional mixing. After 45 min the poly(rI)·poly(rC)-Sepharose was removed by centrifugation, and the supernatant solution was treated with micrococcal nuclease (240 units/ml for 2.5 min at room temperature) to remove small amounts of dsRNA (1–2 $\mu$g/ml) that had been released from the poly(rI)·poly(rC)-Sepharose. Treatment of unadsorbed extracts of reovirus-infected cells with micrococcal nuclease had no effect on kinase inhibitory activity (data not shown). Adsorbed, nuclease-treated extract from reovirus-infected cells was then mixed with extract from IFN-treated cells, and the mixture was assayed for protein phosphorylation.

## RESULTS

**P$_1$ and eIF-2α Protein Kinase Activity in S-10 Extracts.** Extracts prepared from mock treated L cells exhibit a low level of P$_1$ and eIF-2α phosphorylation in the presence of an

optimal concentration of dsRNA (1 $\mu$g/ml) (Fig. 1, lane E; ref. 4). Treatment with mouse IFN increases the level of P$_1$ phosphorylation ≈5-fold (Fig. 1, lane C; ref. 1). However, we were unable to detect any dsRNA-stimulated phosphorylation of P$_1$ or eIF-2α in extracts prepared from reovirus serotype 1-infected cells (Fig. 1, lane A). Extracts prepared from reovirus-infected cells were also unable to phosphorylate exogenously added histone protein substrate (Fig. 1, lane G). These results suggest that kinase was either not present or not assayable in these extracts.

**Reovirus Serotype 1-Infected Cells Contain an Inhibitor of the dsRNA-Activated Protein Kinase.** To determine if the inability to detect the dsRNA-dependent phosphorylation of P$_1$ and eIF-2α in extracts prepared from reovirus serotype 1-infected cells might be due to the presence of an inhibitor, extracts from reovirus-infected cells were mixed with extracts prepared from IFN-treated cells prior to assaying for kinase. The results (Fig. 2) demonstrate that extracts prepared from reovirus serotype 1-infected cells inhibited the dsRNA-dependent phosphorylation of P$_1$ in extracts prepared from IFN-treated cells (Fig. 2, lane A). This suggests that reovirus-infected cells contain a diffusible inhibitor of the IFN-induced protein kinase.

**Reversal of Protein Kinase Inhibitor Effect by High Concentrations of dsRNA.** The IFN-induced, dsRNA-dependent protein kinase can be activated with either reovirus dsRNA or poly(rI)·poly(rC) present at 0.01–0.1 $\mu$g/ml (26). One microgram of dsRNA per ml is normally optimal for kinase activation in extracts prepared from either untreated (Fig. 3A) or IFN-treated cells (Fig. 3B), with concentrations higher than 10 $\mu$g/ml being suboptimal for activation (4). Although no activation of kinase could be detected in extracts from reovirus-infected cells in the presence of poly(rI)·poly(rC) at a concentration of 1 $\mu$g/ml, incubation with a concentration of 10 $\mu$g/ml led to kinase activation, as detected by P$_1$ and eIF-2α phosphorylation (Fig. 3C, lane F). This concentration of dsRNA, required to activate kinase in extracts of reovirus-infected cells, is ≈100-fold greater than the concentration of dsRNA required to detectably activate the kinase in uninfected cells (Fig. 3 A and B). A similar fold increase in the dsRNA concentration required for activation of enzyme was seen when histone proteins were used as exogenous substrate (data not shown).

The level of kinase activity in extracts prepared from untreated reovirus-infected cells (Fig. 3C, lane F), as detected by the amount of P$_1$ phosphorylation, was greater than
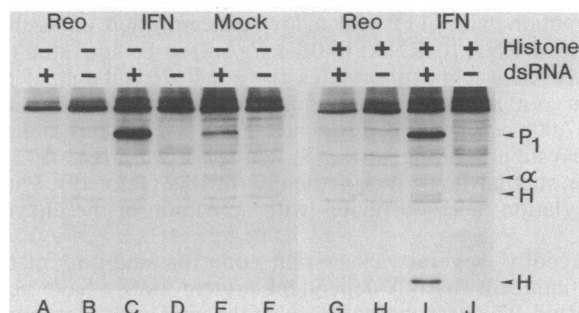


FIG. 1. dsRNA-dependent phosphorylation of proteins is not detectable in extracts prepared from reovirus-infected cells. Phosphorylation of proteins *in vitro*, in extracts prepared from reovirus (Reo)-infected cells (lanes A, B, G, and H), IFN-treated, uninfected cells (lanes C, D, I, and J), or untreated, uninfected cells (lanes E and F), was performed in the absence of dsRNA (lanes B, D, F, H, and J) or in the presence of reovirus dsRNA at a concentration of 1 $\mu$g/ml (lanes A, C, E, G, and I). For reactions performed in the presence of histone proteins (lanes G–J), extracts were preincubated with dsRNA prior to adding histone proteins to the reaction mixtures. α, Small subunit of eIF-2; H, histone proteins.
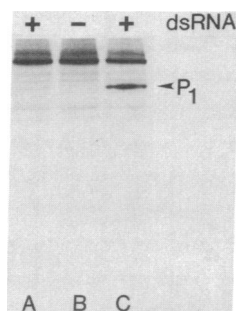
FIG. 2. Extracts prepared from reovirus-infected cells prevent phosphorylation of the dsRNA-dependent protein kinase ($P_1$) in extracts from IFN-treated cells. Extracts prepared from reovirus-infected cells were mixed with an equal amount of extract from IFN-treated cells, and protein phosphorylation was performed *in vitro*, in the presence of dsRNA at a concentration of 1 µg/ml (lane A). As a control, extracts from IFN-treated cells and from infected cells were mixed after protein phosphorylation was performed *in vitro* in the absence (lane B) or presence (lane C) of dsRNA.



FIG. 4. Kinase inhibitory activity in extracts from IFN-treated, reovirus-infected cells can be overcome by increased concentrations of dsRNA. Phosphorylation was performed *in vitro* in detergent extracts prepared from cells pretreated with IFN and then infected with reovirus serotype 1 in the presence of the following concentrations of dsRNA [poly(rI)·poly(rC)]: no dsRNA (lane A), 0.01 µg/ml (lane B), 0.1 µg/ml (lane C), 1 µg/ml (lane D), 10 µg/ml (lane E). Symbols are the same as in Fig. 1.

that seen in extracts prepared from uninfected, non-IFN-treated cells (Fig. 3A) but less than that seen in extracts prepared from uninfected, IFN-treated cells (Fig. 3B). This suggests that synthesis of this kinase has been induced during virus-infection, perhaps by endogenous synthesis of IFN.

Kinase inhibitory activity could also be detected in extracts prepared from cells pretreated with IFN and then infected with reovirus serotype 1 (Fig. 4). Inhibitory activity could be overcome by incubating extracts in the presence of poly(rI)·

poly(rC) at a concentration of 1 µg/ml (Fig. 4, lane D), a concentration ≈10 times greater than that needed to detectably activate kinase in extracts prepared from uninfected cells (Fig. 3 *A* and *B*).

**Characterization of Inhibitory Activity in Extracts Prepared from Reovirus-Infected Cells.** We have recently found (32) that histone proteins act as inhibitors of the IFN-induced protein kinase by binding to dsRNA. Since the reovirus σ3 protein is a well-characterized dsRNA-binding protein (20), we decided to investigate whether the inhibitor in reovirus-infected cells might be the σ3 protein. Extracts prepared from reovirus serotype 1-infected cells were treated with mono-specific anti-σ3 immunoglobulin adsorbed to fixed *S. aureus* cells, and the bound proteins were removed by centrifugation. The supernatant solution was used in protein phosphorylation reactions with varying concentrations of dsRNA. The resulting autoradiogram is shown in Fig. 5. The dsRNA concentration required for $P_1$ phosphorylation in extracts prepared from reovirus-infected cells was decreased by a factor of ≈10 by adsorption with immobilized anti-σ3 serum (lanes A–D) compared to extracts adsorbed with serum from an unimmunized animal (lanes E–H).

Anti-σ3 antibody adsorption of extracts of reovirus-infected cells results in removal of µl and µlC in addition to σ3 (data not shown; ref. 27) due to complex formation between
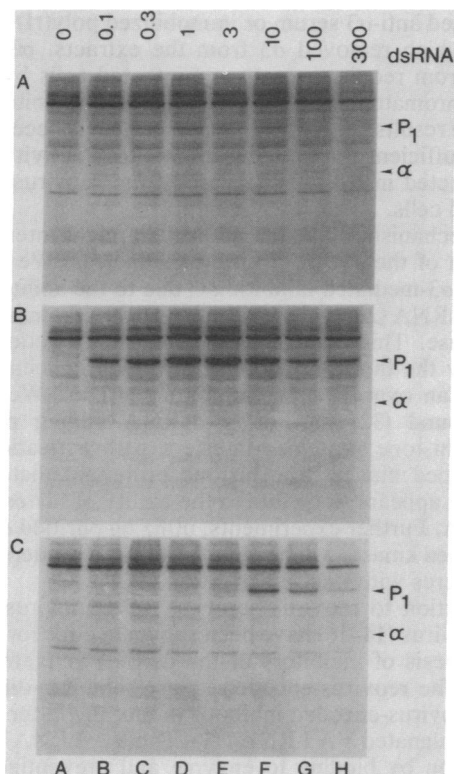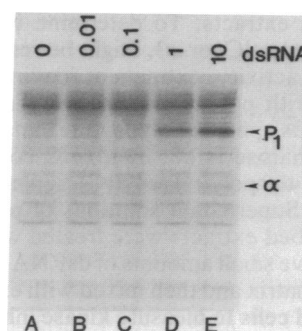


FIG. 3. Kinase inhibitory activity in extracts from reovirus-infected cells can be overcome by increased concentrations of dsRNA. Phosphorylation was performed *in vitro* in extracts prepared from untreated cells (*A*), IFN-treated cells (*B*), or reovirus-infected cells (*C*) in the presence of the following concentrations of dsRNA [poly(rI)·poly(rC)]: no dsRNA (lane A), 0.1 µg/ml (lane B), 0.3 µg/ml (lane C), 1 µg/ml (lane D), 3 µg/ml (lane E), 10 µg/ml (lane F), 100 µg/ml (lane G), 300 µg/ml (lane H). Symbols are the same as in Fig. 1.
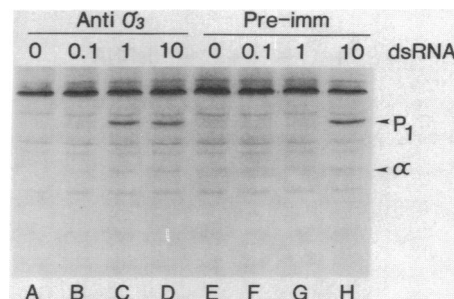


FIG. 5. Adsorption of extracts from reovirus-infected cells with immobilized anti-σ3 serum removes kinase inhibitory activity. Extracts from reovirus-infected cells were incubated with fixed *S. aureus* cells that had been preincubated either with antiserum to the reovirus σ3 protein (lanes A–D) or with serum from an unimmunized rabbit (lanes E–H). Adsorbed proteins were removed by centrifugation, and proteins in the supernatant solutions were phosphorylated *in vitro*. Assays were performed in the absence of dsRNA (lanes A and E) or in the presence of the following concentrations of reovirus dsRNA: 0.1 µg/ml (lanes B and F), 1 µg/ml (lanes C and G), 10 µg/ml (lanes D and H). Symbols are the same as in Fig. 1.

these proteins in extracts. To determine which of the removed proteins, $\mu$l, $\mu$lC, or $\sigma$3, might be responsible for the kinase inhibitory activity, extracts of reovirus-infected cells were adsorbed with poly(rI)·poly(rC)-Sepharose. The only detectable reovirus-specific polypeptide that adsorbs to poly-(rI)·poly(rC)-Sepharose is $\sigma$3; $\sigma$3·$\mu$l/$\mu$lC complexes do not appear to bind to poly(rI)·poly(rC)-Sepharose (data not shown; ref. 20). Supernatant solutions of poly(rI)·poly(rC)-Sepharose-adsorbed extracts were treated with micrococcal nuclease to remove small amounts of dsRNA (1–2 $\mu$g/ml) that eluted from the matrix and then mixed with extracts prepared from IFN-treated cells to measure kinase inhibitory activity. Fig. 6 is an autoradiogram showing the results of phosphorylation reactions with these mixtures. Phosphorylation of $P_1$ could be detected in poly(rI)·poly(rC)-adsorbed extracts after addition of dsRNA to a concentration of 1 $\mu$g/ml (Fig. 6, lane C), a concentration of dsRNA that is decreased by a factor of ≈10 compared to the concentration of dsRNA required for $P_1$ phosphorylation in unadsorbed extracts (Fig. 3$C$, lane F). These results suggest that $\sigma$3 protein is required for kinase inhibitory activity.

We have also purified $\sigma$3 protein by dsRNA affinity chromatography from extracts of reovirus serotype 1-infected cells (20). Kinase inhibitory activity in these preparations was tested by incubation with poly(rI)·poly(rC), purified histones, and partially purified kinase prepared from IFN-treated mouse L cells. Phosphorylation of histone proteins was quantitated by scanning densitometry. In the absence of $\sigma$3, histone phosphorylation was stimulated 2.5-fold by the lowest concentration of dsRNA tested, 0.01 $\mu$g/ml (Fig. 7, lane D) compared to incubation without dsRNA (Fig. 7, lane B). Addition of dsRNA to a concentration of 0.1 $\mu$g/ml (Fig. 7, lane F) led to a 13-fold increase in histone phosphorylation, whereas addition of dsRNA to a concentration of 1 $\mu$g/ml led to a 100-fold increase (Fig. 7, lane H). In the presence of $\sigma$3, no stimulation of histone phosphorylation occurred until dsRNA was added to a concentration of 1 $\mu$g/ml (Fig. 7, lane G). At this concentration of dsRNA, histone phosphorylation was inhibited 80% compared to reactions lacking $\sigma$3 (Fig. 7, compare lanes G and H). Marked inhibition of $P_1$ phosphorylation was also seen when affinity-purified $\sigma$3 was assayed with partially purified kinase in the absence of histones (data not shown) and when $\sigma$3 was purified by NaDodSO$_4$/PAGE, renatured, and assayed with crude extract from IFN-treated cells (data not shown).



FIG. 7. Purified $\sigma$3 protein has kinase inhibitory activity. Phosphorylation of purified histone proteins by partially purified kinase was performed in the absence of dsRNA (lanes A and B) or in the presence of poly(rI)·poly(rC) at the following concentrations: 0.01 $\mu$g/ml (lanes C and D), 0.1 $\mu$g/ml (lanes E and F), 1 $\mu$g/ml (lanes G and H). Reactions for lanes A, C, E, and G contained 2–5 $\mu$g/ml of $\sigma$3; reactions for lanes B, D, F, and H had no further additions. Symbols are the same as in Fig. 1.

## DISCUSSION

In this paper we demonstrate that extracts prepared from reovirus serotype 1-infected mouse L cells contain an inhibitor of the IFN-induced, dsRNA-activated protein kinase. This inhibitor was functional in assays containing mixed extracts of IFN-treated and reovirus-infected L cells. Inhibitor could be overcome by adding ≈100-fold excess of dsRNA over the amount required for activation of enzyme in extracts prepared from uninfected cells. Inhibitory activity could be removed by adsorption of extracts with either immobilized anti-$\sigma$3 serum or immobilized poly(rI)·poly(rC), both of which removed $\sigma$3 from the extracts. $\sigma$3 protein, purified from reovirus serotype 1-infected cells by dsRNA affinity chromatography, possessed kinase inhibitory activity. These results suggest that the $\sigma$3 protein is necessary and may be sufficient for the kinase inhibitory activity that we have detected in extracts prepared from reovirus serotype 1-infected cells.

The mechanism by which $\sigma$3 protein might interfere with activation of the $P_1$/eIF-2 kinase in unclear. We favor the idea that $\sigma$3-mediated inhibition is due to the ability of $\sigma$3 to bind to dsRNA (20) and prevent the dsRNA from interacting with kinase. This postulated mechanism of action is supported by the fact that addition of a high concentration of dsRNA can overcome the inhibitory effects. We have recently found (32) that other dsRNA-binding proteins—namely, histone proteins—interfere with activation of the IFN-induced kinase. The histone protein-mediated kinase inhibition appears to be due to the ability of histone to bind to dsRNA. Further experiments, utilizing purified $\sigma$3 protein and purified kinase, will be required to better understand how $\sigma$3 interferes with kinase activation.

In addition to reovirus serotype 1, adenovirus (11) and vaccinia virus (14–16) have been shown to code for or induce the synthesis of inhibitors of the dsRNA-activated protein kinase. The reovirus-encoded kinase inhibitor differs from the adenovirus-encoded inhibitor in that the latter is a small RNA, designated VA I RNA (11). The VA I RNA is thought to function by binding to enzyme and preventing enzyme from binding to dsRNA (12). Thus, although the mode of action of these two inhibitors is likely different, the outcome—interference with binding of enzyme to dsRNA—may be the same. The mode of action and physical nature of the vaccinia virus-encoded kinase inhibitor are not well characterized. The vaccinia virus inhibitor is thought to be a protein that interacts with dsRNA in a stoichiometric manner (17). It is possible, therefore, that the vaccinia virus inhibitor may function in a manner similar to the reovirus $\sigma$3 protein.
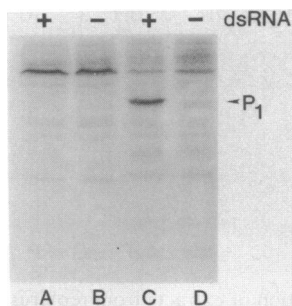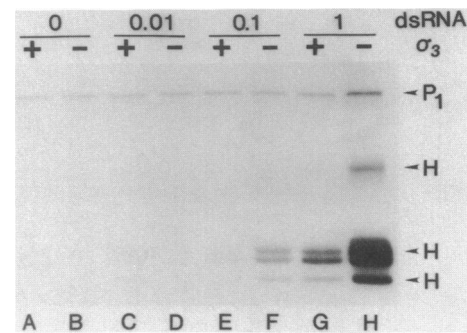


FIG. 6. Adsorption of extracts from reovirus-infected cells with immobilized poly(rI)·poly(rC) removes kinase inhibitory activity. Extracts from reovirus-infected cells either were incubated with poly(rI)·poly(rC)-Sepharose (lanes C and D) or were left untreated (lanes A and B). Poly(rI)·poly(rC)-Sepharose-adsorbed proteins were removed by centrifugation, and the supernatant solutions were assayed for kinase inhibitory activity. Lanes A and C, assays performed in the presence of dsRNA at a concentration of 1 $\mu$g/ml; lanes B and D, assays performed in the absence of dsRNA.

At present we do not know the physiological significance, regarding inhibition of reovirus replication by IFN, of synthesis of kinase inhibitory activity in reovirus serotype 1-infected cells. Reovirus replication is generally considered to be sensitive to treatment of cells with IFN (28). However, our preliminary data suggest that replication of reovirus serotype 3 (Dearing) is considerably more sensitive to IFN treatment than replication of serotype 1 (Lang) (R. Ferguson and B.L.J., unpublished observations). Extracts from reovirus serotype 1-infected cells, prepared at the peak of viral protein synthesis (29), also contain significantly more kinase inhibitory activity and more σ3 protein than similar extracts prepared from reovirus serotype 3-infected cells (unpublished observations). Experiments are necessary to determine if the difference in IFN sensitivity between the two serotypes might be due to the difference in the amount of kinase inhibitory activity synthesized in cells infected with the respective viruses.

Cells infected with the three serotypes of reovirus differ markedly in terms of the timing and level of shutoff of host protein synthesis (29, 30). Host protein synthesis continues unabated in reovirus serotype 1-infected cells, whereas it is shut off rapidly and completely in reovirus serotype 2-infected cells (30). In reovirus serotype 3-infected cells shutoff of host protein synthesis occurs only at late times after infection and to a lesser extent than in serotype 2-infected cells (29, 30). The reovirus σ3 protein has been implicated by genetic studies in the rapid and complete shutoff of host protein synthesis in serotype 2-infected cells (30). This protein has also recently been shown to enhance the translation *in vitro*, in extracts prepared from uninfected cells, of *in vivo* synthesized reovirus mRNA (31). It is possible that the ability of σ3 to modulate protein synthesis *in vivo* and *in vitro* may be mediated by its ability to function as an inhibitor of the dsRNA-activated, eIF-2 kinase.

1. Leubleu, B., Sen, G. C., Shaila, S., Cabrer, B. & Lengyel, P. (1976) *Proc. Natl. Acad. Sci. USA* 73, 3107–3111.
2. Rice, A. P., Duncan, R., Hershey, J. W. B. & Kerr, I. M. (1985) *J. Virol.* 54, 894–898.
3. Samuel, C. E., Duncan, R., Knutson, G. S. & Hershey, J. W. B (1984) *J. Biol. Chem.* 259, 13451–13457.
4. Miyamoto, N. G., Jacobs, B. L. & Samuel, C. E. (1983) *J. Biol. Chem.* 258, 15232–15237.
5. Roberts, W. K., Hovanessian, A. G., Brown, R. E., Clements, M. J. & Kerr, I. M. (1976) *Nature (London)* 264, 477–480.
6. Samuel, C. E., Farris, D. A. & Eppstein, D. A. (1977) *Virology* 83, 56–71.
7. Zilberstein, A., Federman, P., Shulman, L. & Revel, M. (1976) *FEBS Lett.* 68, 119–124.
8. Samuel, C. E., Knutson, G. S., Berry, M. J., Atwater, J. A. & Lasky, S. R. (1986) *Methods Enzymol.* 119, 499–516.
9. Konieczny, A. & Safer, B. (1983) *J. Biol. Chem.* 258, 3402–3408.
10. Hovanessian, A. G., Galabru, J., Svab, J., Laurent, A. G., Robert, N. & Krust, B. (1985) in *The Interferon System 1985*, eds. Dianzani, F. & Rossi, G. B. (Raven, New York), Vol. 24, pp. 151–155.
11. Schneider, R. J. & Shenk, T. (1987) *Annu. Rev. Biochem.* 56, 317–332.
12. Katze, M. G., deCorato, D., Safer, B., Galabru, J. & Hovanessian, A. G. (1987) *EMBO J.* 6, 689–697.
13. Kitajewski, J., Schneider, R. J., Safer, B., Munemitsu, S. M., Samuel, C. E., Thimmapaya, B. & Shenk, T. (1986) *Cell* 45, 195–200.
14. Whitaker-Dowling, P. & Youngner, J. S. (1983) *Virology* 131, 128–136.
15. Rice, A. P. & Kerr, I. M. (1984) *J. Virol.* 50, 229–236.
16. Paez, E. & Esteban, M. (1984) *Virology* 134, 12–28.
17. Whitaker-Dowling, P. & Youngner, J. S. (1984) *Virology* 137, 171–181.
18. Sen, G. C., Taira, H. & Lengyel, P. (1978) *J. Biol. Chem.* 253, 5915–5921.
19. Kimchi, A., Zilberstein, A., Schmidt, A., Shulman, L. & Revel, M. (1979) *J. Biol. Chem.* 254, 9846–9853.
20. Huismans, H. & Joklik, W. K. (1976) *Virology* 70, 411–424.
21. Miyamoto, N. G. & Samuel, C. E. (1980) *Virology* 107, 461–475.
22. Samuel, C. E. (1981) *Methods Enzymol.* 79, 168–178.
23. Neal, M. W. & Florini, J. R. (1973) *Anal. Biochem.* 55, 328–330.
24. Samuel, C. E., Knutson, G. S., Berry, M. J., Atwater, J. A. & Lasky, S. R. (1986) *Methods Enzymol.* 119, 499–516.
25. Ito, Y. & Joklik, W. K. (1972) *Virology* 50, 189–201.
26. Jacobs, B. L., Miyamoto, N. G. & Samuel, C. E. (1988) *J. Interferon Res.*, in press.
27. Lee, P. W. K., Hayes, E. C. & Joklik, W. K. (1981) *Virology* 108, 134–146.
28. Wiebe, M. E. & Joklik, W. K. (1975) *Virology* 66, 229–240.
29. Munemitsu, S. M. & Samuel, C. E. (1984) *Virology* 136, 133–143.
30. Sharpe, A. H. & Fields, B. N. (1982) *Virology* 122, 381–391.
31. Lemieux, R., Lemay, G. & Millward, S. (1987) *J. Virol.* 61, 2472–2479.
32. Jacobs, B. L. & Imani, F. (1988) *J. Interferon Res.*, in press.