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Mechanism of protein synthesis inhibition by vaccinia viral core and reversal of this inhibition by reticulocyte peptide chain initiation factors

DEBOPAM CHAKRABARTI, KOLLURU V. A. RAMAIAH, ANANDA L. ROY, MILAN BAGCHI and NABA K. GUPTA*
Department of Chemistry, The University of Nebraska, Lincoln, Nebraska U.S.A. 68588-0304

Abstract. Vaccinia viral core inhibits protein synthesis in heme-supplemented reticulocyte lysate. A reticulocyte cell supernatant factor, which reversed protein synthesis inhibition in heme-deficient reticulocyte lysate also reversed vaccinia viral core induced protein synthesis inhibition in heme-supplemented reticulocyte lysate. Significant inhibition reversal activity was also observed with a partially purified eukaryotic initiation factor-2 preparation and this activity was lost upon further purification of eukaryotic initiation factor-2.The ribosomal salt-wash factor Co-eukaryotic initiation factor-2 which like reticulocyte supernatant factor contains guanine nucleotide exchange factor activity, was completely inactive. Vaccinia viral core induced detectable level of eukaryotic initiation factor-2 α-subunit phosphorylation when incubated in the heme-supplemented reticulocyte lysate. This lysate preparation contains guanine nucleotide exchange factor activity. However, when the same reticulocyte lysate was previously incubated with the vaccinia viral core, the guanine nucleotide exchange factor activity during subsequent incubation was almost completely inhibited.

Keywords. Vaccinia virus; protein synthesis; peptide chain initiation factor.

Introduction

The infection of animal cells with viruses is often accompanied by shut-off of host protein synthesis. There are indications that some step(s) in peptide chain initiation is involved in this inhibition (Bablanian, 1975). Recently several laboratories have reported that protein synthesis inhibition in cell-free extracts obtained from virus-infected cells could be reversed by the addition of exogeneous peptide chain initiation factors such as eukaryotic initiation factor-2 (eIF-2) (Centralla and Lucas-Lennard, 1982; Dratewka-Kos *et al*., 1984; Reichel *et al*., 1985; Siekerka *et al*., 1985) guanine nucleotide exchange factor (GEF) Dratewka-Kos *et al*., 1984; Reichel *et al*., 1985; Siekerka *et al*., 1985) eIF-4B (Dratewka-Kos *et al*., 1984; Van Steeg *et al*., 1984) and eIF-4F (Centralla and Lucas-Lennard, 1982; Griffo *et al*., 1985; Edery *et al*., 1983) indicating possible alterations of these factor(s) during virus infection.

In the case of vaccinia virus, protein synthesis inhibition occurs in the absence of viral RNA or new protein synthesis (Shatkin, 1965; Moss, 1968; Rosemond-Horntseak

*To whom all correspondence should be addressed.

Abbreviations used: eIF-2, Eukaryotic initiation factor 2, which forms a ternary complex, Met-tRNA$_f$eIF-2-GTP; Co-eIF-2, a high molecular protein complex isolated from ribosomal salt wash; RF, a high molecular weight protein complex isolated from reticulocyte cell supernatant; GEF, guanine nucleotide exchange factor; EMC, encephalomyocarditis virus; HRI, heme-regulated protein synthesis inhibitor.

and Moss, 1975; Moss and Filler, 1970; Bablanian *et al.*, 1978; Schrom and Bablanian, 1979) and the time required for the establishment of shut-off corresponds to the first stage of uncoating *i.e.* the release of cores into the cytoplasm (Joklik, 1969; Dalls, 1965). Using a cell-free system, Ben Hamida and Beaud (1978) have reported that vaccinia viral core inhibits protein synthesis and also Met-tRNA$_f$ 40S initiation complex formation in reticulocyte lysate (Parson *et al.*, 1980). Recently, Coppola and Bablanian (1983) have reported that the vaccinia virus transcripts generated *in vitro* by transcription of viral core, inhibited translation of cellular and encephalomyocarditis virus (EMC) mRNA but not that of vaccinia viral mRNA in reticulocyte lysate.

Several years ago, we reported (Ghosh-Dastidar *et al.*, 1981) that vaccinia viral core inhibits Met-tRNA$_f$·40S initiation complex formation in reticulocyte lysate in response to physiological mRNAs and not in response to AUG codon indicating that viral core inhibits some step(s) in peptide chain initiation involved in recognition of the structural features unique to physiological mRNAs. In this paper, we report that a reticulocyte cell supernatant factor, (RF) and a partially purified eIF-2 preparation which reverse protein synthesis inhibition in heme-deficient reticulocyte lysate (Gross, 1976; Ranu and London, 1977; Amsez *et al.*, 1979; Ralston *et al.*, 1979; Grace *et al.*, 1982, 1984; Siekierka *et al.*, 1981, 1982, 1983; Matts *et al.*, 1983; Konieczny and Safer, 1983) also reverse vaccinia viral core induced protein synthesis inhibition in reticulocyte lysate. The possible mechanism of vaccinia viral core induced protein synthesis inhibition and its reversal by RF have been discussed.

A preliminary report of this work has been presented (Chakrabarti *et al.*, 1985).

Materials and methods

The sources of most of the materials used in these studies were the same as described previously (Ghosh-Dastidar *et al.*, 1980, 1981; Ralston *et al.*, 1979; Grace *et al.*, 1982, 1984; Majumdar *et al.*, 1979; Das *et al.*, 1982; Chakravarty *et al.*, 1985; Bagchi *et al.*, 1984). [^{35}S]-Methionine (1490 Ci/mmol) was obtained from Amersham/Searle. [^{3}H]-GDP (13 Ci/mmol) and [^{14}C]-Leucine (344 mCi/mmol) were obtained from New England and Nuclear and [γ^{32}P]-ATP (4500 Ci/mmol) was purchased from ICN.

Reticulocyte ribosomes, ribosomal subunits and peptide chain initiation factors

Reticulocyte ribosomes, 40S ribosomal subunits and ribosomal salt (0·5 M KC1) wash were prepared as described previously (Chakravarty *et al.*, 1985). Partially purified eIF-2 (Fraction III) and Co-eIF-2 preparations were obtained after a CM-Sephadex chromatography step, as described previously (Das *et al.*, 1982). The eIF-2 activity was further purified using a hydroxylapatite column chromatographic procedure (Fraction IV). The hydroxylapatite column was equilibrated with Buffer A containing 10 mM potassium phosphate, 10% glycerol, 5 mM ß-mercaptoethanol and 50 μM EDTA. The Fraction III eIF-2 preparation was applied onto the column. The column was washed with 3 column volumes of Buffer A containing 200 mM potassium phosphate (pH 7·8) and the proteins were then eluted from the column using a potassium phosphate (pH 7·8) gradient (200 mM→500 mM) in Buffer A. The

eIF-2 activity eluted at potassium phosphate concentration range around 300 mM. The fractions showing peak eIF-2 activity were pooled, and the pooled fraction was dialyzed against a buffer containing 20 mM Tris-HCl, 10% glycerol, 5 mM ß-mercaptoethanol and 50 μM EDTA. The dialyzed solution was concentrated by Centricon micro concentrator and stored in small aliquots in liquid nitrogen (Fraction IV).

The Co-eIF-2 activity after CM-Sephadex chromatography was further purified using DEAE-cellulose chromatography (Das *et al*., 1982). The reticulocyte cell supernatant factor RF was purified following the procedure of Grace *et al*. (1984). As before, Fraction V RF preparation contained significant amounts of eIF-2 activity and the bulk of this eIF-2 activity was removed at the next purification step using CM-Sephadex column chromatography (Fraction VI).

Preparation of viral cores

Vaccinia virus was isolated and purified according to the method of Joklik (1962) and Nevins and Joklik (1977). Viral core was isolated and purified as described before (Ghosh-Dastidar *et al*., 1981). Purified cores were resuspended by sonication in 10 mM Tris-HCl, pH 8·4.

Preparation of rabbit reticulocyte lysates and assay of protein synthesis

The procedures for the preparation of reticulocytes and reticulocyte lysates have been described (Ghosh-Dastidar *et al*., 1980, 1981). Protein synthesis was assayed by the incorporation of [^{14}C]-Leucine into hot trichloroacetic acid-insoluble protein.

Peptide chain initiation assays

eIF-2 and GEF activity in different factor preparations were assayed using the standard millipore filtration assay method as described previously (Chakravarty *et al*., 1985; Bagchi *et al*., 1984).

Details of the individual experimental procedures have also been described.

Gel Electrophoresis

$NaDodSO_4$ /polyacrylamide gel electrophoresis was performed as described by Laemmli (1970) using a 10% acrylamide/0·16% N,N"methylenebisacrylamide gel. Autoradiograms were made with Kodak X-OMAT AR X-ray film.

Results

We studied the effects of addition of vaccinia viral core and the reticulocyte cell supernatant factor, RF on protein synthesis in reticulocyte lysate (figure 1). In these experiments, the heme-supplemented reticulocyte lysate was preincubated with or without the viral core for 10 min at 24°C and protein synthesis was then determined

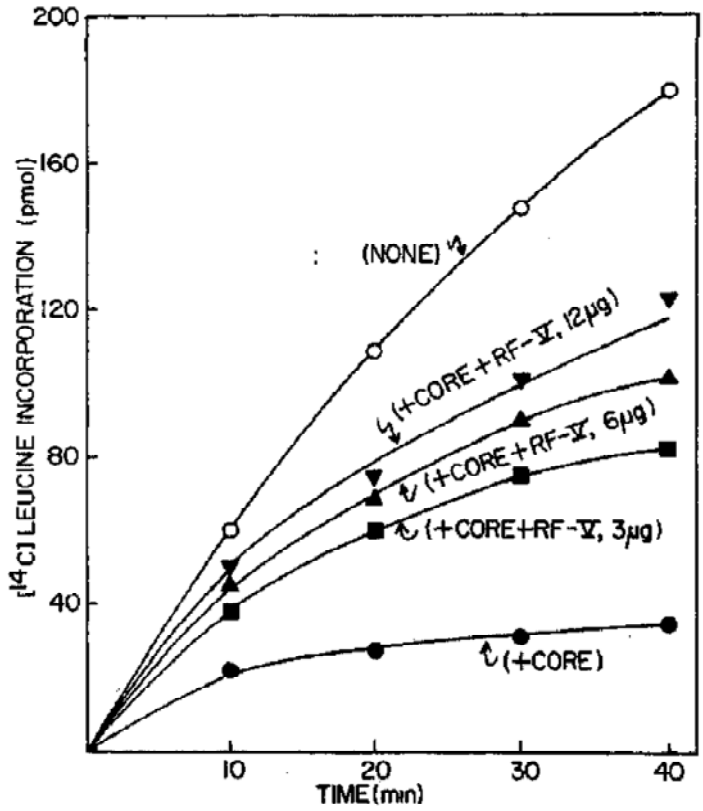

Figure 1. Effects of addition of vaccinia viral core and the reticulocyte cell supernatant factor (RF, Fraction V) on protein synthesis in reticulocyte lysate.

The reaction mixture containing 15 μl reticulocyte lysate, 75 mM KC1, 37 μM Hemin-Cl was preincubated at 25°C for 10 min with 0·11 A_{260} unit of vaccinia viral core as indicated. The reaction mixture (final volume 45 μl) was then mixed with 37·5 μM amino acid mixture (-Leu), 38 μM [^{14}C]-Leucine incorporation in 20 μl aliquot was determined by standard method.

after further addition of protein synthesis components and RF as indicated. The reticulocyte lysate efficiently incorporated [14C]-Leucine and the addition of excess RF marginally stimulated such activity. However, preincubation with the viral core, reduced the protein synthesis activity of the lysate to approximately 20%. Addition of RF significantly reversed this viral core induced protein synthesis inhibition and this reversal activity increased with increasing RF concentration-. Approximately, 70% of the original protein synthesis activity of the lysate was restored in the presence of 12 μg, RF.

We compared the activities of several factor preparations, such as RF (Fraction V and VI) Co-eIF-2 and eIF-2, for reversal of viral core induced protein synthesis inhibition in reticulocyte lysate (table 1). RF V fraction contained significant amounts of both eIF-2 and GEF activities and bulk of the eIF-2 activity was removed by further purification using CM-Sephadex chromatography (RF Fraction VI). Fraction VI RF preparation was enriched in GEF activity. The ribosomal salt wash factor Co-eIF-2 does not reverse protein synthesis inhibition in heme-deficient reticulocyte lysate but contains significant amount of GEF activity. As shown here

Table 1. Activities of different factor preparations for reversal of vaccinia viral core induced protein synthesis inhibition in reticulocyte lysate.

Factor(s) Added	Amount (μg)	[^{14}C]-Leu incorporated pmol/20 μl incubation mixture	Activity (%)
None	—	240	100
Viral core	—	60	25
Viral core + RF V	4	140	58
Viral core + RF VI	4	100	42
Viral core + Co-eIF-2	40	70	29
Viral core + eIF-2 III	4	120	50
Viral core + eIF-2 IV	8	70	29

Heme-supplemented reticulocyte lysate was incubated with 0·11A260 unit vaccinia viral core for 10 min at 25°C. Amino acid incorporation into protein was then determined using standard assay conditions.

(table 1), both Fraction V and VI RF preparations reversed viral core induced protein synthesis inhibition although ternary complex activity of Fraction VI RF preparation was significantly less. The Co-eIF-2 preparation even at 40 μg concentration did not have any significant effect on protein synthesis. A partially purified eIF-2 preparation (Fraction III approximately 50% pure as judged by sodium dodecyl sulphate-gel) showed significant protein synthesis inhibition reversal activity. However, upon further purifications using a hydroxylapatite chromatography (Fraction IV), this protein synthesis inhibition reversal activity was lost. The purity of the Fraction IV eIF-2 preparation is over 80% and this fraction shows mainly 3 polypeptide bands characteristic of eIF-2.

To determine the component activity or activities in RF and eIF-2 preparations responsible for reversal of viral core induced protein synthesis inhibition, we analyzed the GEF and eIF-2 activities in these preparations (tables 2 and 3). As shown in table 2, the GEF activity in both Fraction V and VI RF preparation was comparable whereas the GEF activity in Co-eIF-2 preparation was significantly less (approximately 10% of that observed with Fraction V and VI RF preparations). The eIF-2 preparations (Fraction III and IV) showed no detectable level of GEF activity (data not shown here). It should be emphasized that the experiments described in table 2 were performed using RF V, VI and Co-eIF-2 preparations containing comparable amounts of GEF activity. As shown in tables 1 and 2, there is no direct correlation between GEF activity and protein synthesis inhibition reversal activity.

The eIF-2 activities in different factor preparation are shown in table 3. The eIF-2 activities were assayed in the absence of Mg^{2+} and in the presence of excess Co-eIF-2 (Grace *et al.*, 1984). Co-eIF-2 preparations were completely devoid of eIF-2 activity. As shown here Fraction V RF preparation contains some eIF-2 activity and Fraction VI RF preparation is almost devoid of eIF-2 activity. The partially purified eIF-2 preparation (eIF-2 III) was fully active in ternary complex formation. Almost 100% of the potentially active eIF-2 molecules formed ternary complex (this factor preparation is 50% pure and 1·5 μg protein formed 5·0 pmol ternary complex). Upon further purification using hydroxylapatite chromatography, the eIF-2 was

Table 2. Comparison of GEF activity in different factor preparations.

Factor	Amount (μg)	[^{3}H]-GDP displaced (%)
RF-V	1·0	46
	2·0	58
RF-VI	1·0	50
	2·0	63
Co-eIF-2	10·0	41
	20·0	59

eIF-2·[^{3}H]-GDP was preformed (5 min, 37°) in a reaction mixture (total vol., 50 μl) containing 25 mM Tris-HCl (pH 7·8), 90 mM KC1, 2·5 mM dithiothreitol, bovine serum albumin, 160 μg/ml; eIF-2 1·5μg and 1·5μM [^{3}H]-GDP (5400 cpm/pmol). After the incubation $Mg(OAc)_2$ and unlabelled GDP was added to a final concentration of 1 mM and 100 μM respectively. eIF-2·[^{3}H]-GDP (50 μl) and different amount of factor preparation was incubated at 37°C for 15 seconds. Ice-cold wash buffer (3 ml) containing 20 mM Tris-HCl (pH 7·8), 100 mM KCl and 1 mM $Mg(OAc)_2$ was added to stop the reaction. The reaction mixtures were then assayed using standard Millipore filtration conditions for [^{3}H]-GDP release.

Table 3. Ternary complex formation by different factor preparations.

Factor	[^{35}S] Met-tRNA$_f$ bound (pmol)
RF V	1·0
RF VI	0·1
eIF-2 III	5·0
eIF-2 IV	2·3

Standard Millipore filtration assay conditions were used in the presence of 15 μg Co-eIF-2. Amounts of different factors used were 1·5 μg each.

freed of contaminating proteins. However, as reported earlier, extensively purified eIF-2 preparations looses activity rapidly upon storage, and as shown in table 3, approximately 25-30% of the potentially active eIF-2 molecules formed ternary complexes. As reported in table 1, RF V, VI and eIF-2 preparations reversed viral core induced protein synthesis inhibition. The results presented in table 3, clearly demonstrate that there is no direct correlation between the eIF-2 activity and the protein synthesis inhibition reversal activity. The eIF-2 III preparation is completely devoid of GEF activity but actively reversed viral core induced protein synthesis

inhibition. Clearly, this reversal activity in eIF-2 preparation is not related to GEF activity. This activity was completely lost upon further purification of eIF-2 using hydroxylapatite chromatography (Fraction IV). Fraction IV eIF-2 is foully active in several partial reactions studied including ternary and Met-tRNA$_f$ ·40S complex formation. The characteristics of the protein synthesis inhibition reversal activity in eIF-2 III preparation is not clear. Apparently, the presence of eIF-2 activity cannot explain the protein synthesis inhibition reversal activities in RF Fractions V and VI. As shown in table 3, RF Fraction VI is almost completely devoid of eIF-2 activity.

Because of the involvement of RF in protein synthesis inhibition reversal, we considered the possibility of eIF-2 α-subunit phosphorylation during this inhibition. The eIF-2 α-subunit phosphorylation was compared in both heme-deficient reticulocyte lysate and also in heme-supplemented lysate in the presence of viral core (figure 2). Exogeneous eIF-2 was used in these experiments. In both cases, there was significant increase in eIF-2 α-subunit phosphorylation over control experiments; heme-deficient lysate (figure 2, lane 3) *vs* heme-supplemented lysate (figure 2, lane 2) and heme-supplemented lysate with viral core (figure 2, lane 5) *vs* heme-supplemented lysate without viral core (figure 2, lane 4). However, the extent of the eIF-2 phosphorylation in both cases was significantly less when compared to the extent of phosphorylation of added eIF-2 by exogeneously added heme-regulated protein synthesis inhibitor (HRI) (figure 2, lane 1). We should point out, however, that in the absence of exogeneously added eIF-2, we did not detect any significant phosphorrylation of eIF-2 either in heme-deficient reticulocyte lysate or in heme-supplemented lysate with viral core although under both conditions the protein synthesis activity of the lysate was strongly inhibited. Apparently, the low sensitivity of our assay procedure precluded detection of eIF-2 phosphorylation under these conditions.

An indirect evidence for viral core-induced eIF-2 α-subunit phosphorylation was also obtained by assaying the GEF activity in an aliquot of reticulocyte lysate used for protein synthesis. It has been reported that eIF-2 α(P) formed by phosphorylation of eIF-2 α-subunit by HRI binds to GEF and inactivates it. As shown in figure 3, the reticulocyte lysate used for protein synthesis activity promotes [^{3}H]- GDP displacement from exogeneously added eIF-2·[^{3}H]- GDP in the presence of unlabelled GDP and this GDP displacement activity was almost completely lost upon prior incubation of the lysate with vaccinia viral core presumably by inactivation of endogeneous GEF by eIF-2 α (P) formed during incubation with the viral core.

Discussion

Vaccinia viral core inhibits protein synthesis and also Met-tRNA$_f$·40S complex formation with physiological mRNAs in reticulocyte lysate (Ghosh-Dastidar *et al.*, 1981). In this paper, we provide evidence that vaccinia viral core promotes limited eIF-2 α-subunit phosphorylation when incubated in the presence of reticulocyte lysate and exogeneously added eIF-2 and also inhibits the endogeneous GEF activity in reticulocyte lysate. The viral core induced eIF-2 α-subunit phosphorylation may be the cause of viral core induced protein synthesis inhibition in reticulocyte lysate. Several laboratories have now reported eIF-2 α-subunit phosphorylation under different physiological conditions, such as virus infection

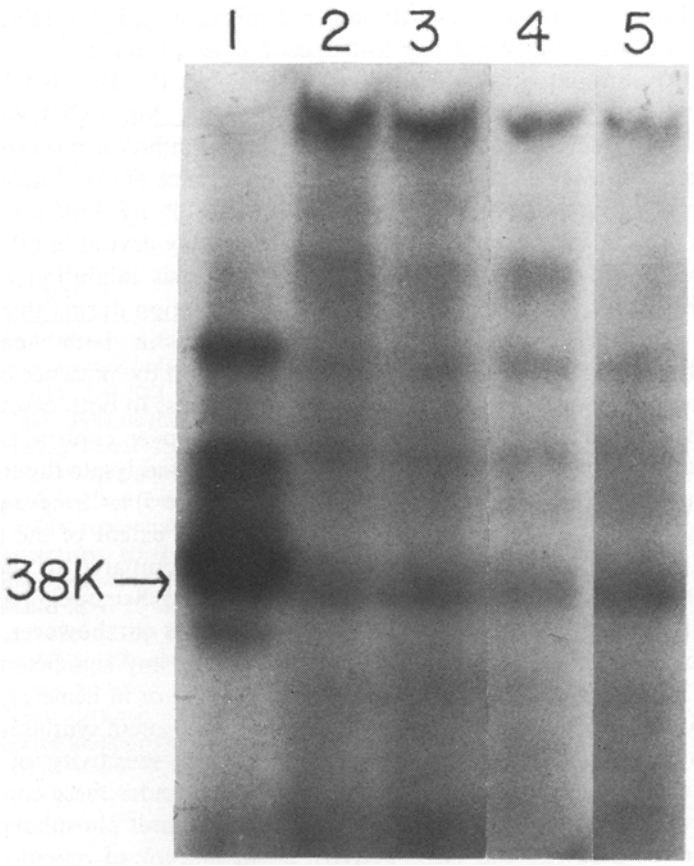

Figure 2. Phosphorylation of eIF-2 α-subunit.

The reaction mixtures contained 15 μl reticulocyte lysate, 75 μM KCl, 37 μM Hemin-Cl, 8 μg eIF-2 and 0·11A_{260} unit of vaccinia viral core as indicated. The reaction mixtures were incubated at 24°C for 10 min and were then mixed with (final vol, 45 μl), 37·5 μM amino acid mixture, 0·15 mM GTP, 7·5 mM creatine phosphate 2·25 U creatine phosphokinase, 10 mM Tris-HCl, pH 7·8. The reaction mixtures were then incubated at 30°C for 40 min. At all stages the reaction mixture contained 0·5 μM [g-^{32}P] -ATP (sp. activity 10^5 cpm/pmol) and 1·5 mM $Mg(OAC)_2$. For *in vitro* eIF-2 phosphorylation by HRI (Lane 1) 8 μg eIF-2 was incubated with 3 μg HRI, [g-^{32}P] -ATP (25 μM; specific activity 2000 cpm/pmol) and 1·5 mM $Mg(OAC)_2$ at 37°C for 10 min. The reaction mixtures were then analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis and autoradiography. Lane 1, eIF-2+ HRI + ATP; Lane 2, eIF-2 + Heme-supplemented lysate; Lane 3, -eIF-2-Heme-supplemented lysate; Lane 4, +eIF-2 + Heme-supplemented lysate-core; Lane 5, +eIF-2 + Hemin supplemented lysate + core.

(Siekierka *et al*., 1985; Van Steeg *et al*., 1984), heat shock conditions (Duncan and Hershey, 1984; DeBenedetti and Baglioni, 1986) and also under different stress conditions (Duncan and Hershey, 1985). In each case, such phosphorylation is accompanied by reduced protein synthesis, or preferential synthesis of specific proteins.

At least two peptide chain initiation factor preparations, RF and eIF-2 (III) reversed protein synthesis inhibition by vaccinia viral core. In a similar study, using

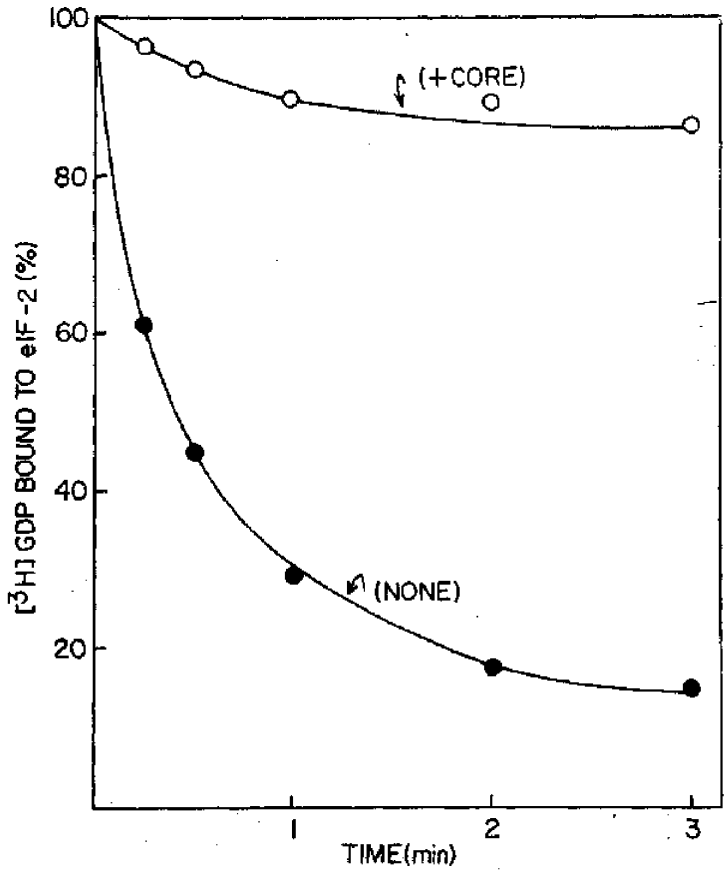

Figure 3. Inhibition of GEF activity in reticulocyte lysate by vaccinia viral core.

The reaction mixture was preincubated at 25°C for 10 min with 150 μl of reticulocyte lysate, 75 mM KC1, 37 μM Hemin-Cl. Vaccinia viral core (50 μl, 0·55 A_{260} unit) were added as indicated. The reaction mixtures were then mixed with 37·5 μM amino acid mixture 21 U creatine phosphokinase, 0·75 mM ATP, 0·15 mM GTP, 7·5 mM creatine phosphate, 1·5 mM (Mg $OAc)_2$, 10·0 mM Tris-HCl, pH 7·8 and 75 mM KC1 and incubated for 40 min at 30°C. The lysate mixture (425 μl) was then diluted 1:1 with lysate dilution buffer containing 40 mM Tris-HCl (pH 7·8), 100 mM KC1, 50 mM KF, 10% Glycerol and 100 μM unlabelled GDP. eIF-2·[^{3}H]-GDP was preformed (5 min, 37°C) in a separate reaction mixture containing 25 mM Tris-HCl (pH 7·8), 90 mM KC1, 2·5 mM dithiothreitol, 160 μg/ml bovine serum albumin, 1·5 μM [^{3}H]-GDP (5400 cpm/pmol) and eIF-2 (30 μg/ml). $Mg(OAc)_2$ was then added to a final concentration of 1 mM. 150 μl of preformed eIF-2[^{3}H]-GDP was incubated with 600 μl of diluted lysate at 37°C. At indicated time 100 μl sample was withdrawn and immediately added to 3 ml of ice-cold wash buffer containing 20 mM Tris-HCl (pH7·8), 100 mM KC1, and 1 mM $Mg(OAc)_2$ to stop the reaction and the [^{3}H]-GDP release was determined by standard Millipore filtration method.

adenovirus infected cell-free extracts, Siekierka *et al*. (1985) have reported that the GEF activity is responsible for reversal of protein synthesis inhibition caused by eIF-2 α-subunit phosphorylation by eIF-2 kinase (Reichel *et al*., 1985). In this work, we have provided evidence that GEF activity is not responsible for reversal of vaccinia viral core induced protein synthesis inhibition in reticulocyte lysate. We have previously reported that RF reverses protein synthesis inhibition in heme-

deficient reticulocyte lysate. An active RF preparation contains an excess eIF-2 α-subunit. We have postulated that RF provides this excess eIF-2 α-subunit to eIF-2 α(P) and thus reconstitute active eIF-2 molecules (Grace *et al*., 1984). We believe, a similar mechanism is operative in RF promoted reversal of vaccinia viral core induced protein synthesis inhibition in reticulocyte lysate. The mechanism of eIF-2 action in reversal of viral core induced protein synthesis inhibition is not apparent. This reversal activity was observed with a partially purified eIF-2 preparation (Fraction III) and this activity was lost upon further purification. Several possibilities may be considered: (i) The reversal activity may be due to some contaminating factor which is lost upon further fractionation. (ii) The reversal activity is due to eIF-2 in association with other component(s) and this component(s) is lost upon further fractionation. (iii) eIF-2 is active in a specific conformation and this conformation is altered upon further fractionation.

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References

Amsez, H., Golmand, S. H., Haudrich-Morre, T., Voorma, H. O. and Benne, R. (1979) *Eur. J. Biochem*., **98**, 513.

Bablanian, R. (1975) *Prog. Med. Virol*, **19**, 40.

Bablanian, R., Esteban, B., Baxt, B. and Sonnadeno, J. A. (1978) *J. Gen. Virol*., **39**, 391.

Bagchi, M. K., Chakravarty, I., Ahmad, M. F., Nasrin, N., Banerjee, A. C, Olson, C. and Gupta, N. K. (1984) *J. Biol. Chem*., **260**, 6950.

Ben Hamida, F. and Beaud, G. (1978) *Proc. Natl. Acad. Sci. USA*, **75**, 175.

Centralla, M. and Lucas-Lennard, J. (1982) *J. Virol*., **41**, 781.

Chakrabarti, D., Nasrin, N., Ramaiah, K. V. A., Bagchi, M. K. and Gupta, N. K. (1985). *13th Internationals Congress of Biochemistry, Amsterdam*, The Netherlands.

Chakravarty, I., Bagchi, M. K., Roy, R., Banerjee, A. C. and Gupta, N. K. (1985) *J. Biol. Chem*., **260**, 6945.

Coppola, G. and Bablanian, R. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 75.

Dalls, S. (1965) *J. Cell. Biol*, **18**, 51.

Das, A., Bagchi, M. K., Ghosh-Dastidar, P. and Gupta, N. K. (1982) *J. Biol. Chem*., **257**, 1282.

DeBenedetti, A. and Baglioni, C. (1986) *J. Biol. Chem*., **261**, 338.

Dratewka-Kos, E., Kiss, I., Lucas-Lennard, J., Mehta, H. B., Woodley, C.L. and Wahba, A. J. (1984) *Biochemistry*, **23**, 6184.

Duncan, R. and Hershey, J. W. B. (1984) *J. Biol. Chem*., **259**, 11882.

Duncan, R. and Hershey, J. W. B. (1985) *J. Biol. Chem*., **260**, 5493.

Edery, I., Humbelin, M., Darveau, A., Lee, K. A. W., Milburn, S., Hershey, J. W. B., Trachsel, H. and Sonnenberg, N. (1983) *J. Biol. Chem*., **258**, 11398.

Ghosh-Dastidar, P., Yaghmai, B, Das, A., Das, H. K. and Gupta, N. K. (1980) *J. Biol. Chem*., **255**, 365.

Ghosh-Dastidar, P., Yaghmai, B. B. Das, A., Das, P. and Gupta, N. K. (1981) *Biochem Biopys. Res Commun*.**99**,946.

Grace, M., Ralston, R. O., Banerjee, A. C. and Gupta, N. K. (1982) *Proc. Natl. Acad. Sci. USA*, **7**9, 6517.

Grace, M., Bagchi, M. K., Ahmad, M. F., Yeager, T., Olson, C., Chakravarty, I, Nasrin, N., Banerjee, A. C. and Gupta, N. K. (1984) *Proc. Natl Acad. Sci. USA*, **79**, 6517.

Grifo, J. A., Tahara, S. M, Moruan, M. A., Shakin, A. J. and Merrick, W. C. (1985) *J. Biol Chem*., **258,** 5804.

Gross, M. (1976) *Biochem. Biophys. Act*., **447**, 445.

Joklik, W. K. (1962) *Virology*, **18**, 9.
Joklik, W. K. (1969) *J. Mol. Biol*, **8**, 262.
Kaempfer, R. (1982) in *Protein Biosynthesis in Eukaryotes* (ed. R. Perez-Bercoff) (New York and London: Plenum Press) p. 223.
Konieczny, A. and Safer, B. (1983) *J. Biol. Chem*., **258**, 3402.
Laemmli, U. K. (1970) *Nature* (*London*), **227**, 680.
Majumdar, A., Dasgupta, A., Chatterjee, B., Das, H. K. and Gupta, N. K. (1979) *Methods EnzymoL*, **60**, 35.
Matts, R. L., Levin, D. H. and London, J. M. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 2559.
Moss, B. (1968) *J. Virol*, **2**, 1028.
Moss, B. and Filler, R. (1970) *J. Virol*, **5**, 99.
Nevins, J. R. and Joklik, W. K. (1977) *J. Biol. Chem*., **252**, 6930.
Parson, A., Ben Hamida, F. and Beaud, G. (1980) *Nature* (*London*), **287**, 355.
Ralston, R., Das, A., Grace, M., Das, H. K. and Gupta, N. K. (1979) *Proc. Natl. Acad. Set USA*, **76**, 5490.
Ranu, R. S. and London, I. M. (1977) *Fed. Proc. Fed. Am. Soc. Exp. Biol*, 36, 868 (Abstr).
Reichel, P., Merrick, W. C., Siekierka, J. and Mathews, M. B. (1985) *Nature* (*London*), **313**, 196.
Rosemond-Horntseak, H. and Moss, B. (1975) *J. Virol*., **16**, 34.
Schrom, M. and Bablanian, R. (1979) *Virology*, **99**, 319.
Shatkin, A. J. (1965) *Nature* (*London*), **199**, 357.
Siekierka, J., Mitsui, K. and Ochoa, S. (1981) *Proc. Natl. Acad. Sci. USA*, **78**, 220.
Siekierka, J, Mauser, L. and Ochoa, S. (1982) *Proc. Natl Acad. Sci. USA*, **79**, 2537.
Siekierka, J, Manne, V, Mauser, L. and Ochoa, S. (1983) *Proc. Natl Acad. Sci. USA*, **80**, 1232.
Siekierka, J, Mariano, T. M., Reichel, P. A. and Mathews, M. B. (1985) *Proc. Natl Acad. Sci. USA*, **82**, 1959.
Van Steeg, H., Kasperatitis, M, Voorma, H. O. and Benne, R. (1984) *Eur. J. Biochem*., **138**, 473.