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Translational control by adenovirus: Lack of virus-associated RNA_I during adenovirus infection results in phosphorylation of initiation factor eIF-2 and inhibition of protein synthesis

(small viral RNA/guanine nucleotide exchange/translational inhibition)

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ABSTRACT **The dl331 mutant of adenovirus serotype 5 fails to produce virus-associated (VA) RNA_I, and cells infected with this mutant do not synthesize proteins efficiently at late times in infection. The translational defect occurs at the level of polypeptide chain initiation, and cell-free extracts prepared from dl331-infected cells exhibit the defect observed *in vivo*. Addition of either eukaryotic initiation factor 2 (eIF-2) or guanine nucleotide exchange factor (GEF) to these cell-free extracts restores translational activity, with GEF functioning more efficiently in this regard. These results suggest that cells infected with the dl331 mutant develop a translational block at the level of GEF-catalyzed guanine nucleotide exchange and that this block is most likely established through phosphorylation of the α subunit of eIF-2. In the present investigation we show that endogenous HeLa cell GEF activity is significantly reduced in cells infected with the dl331 mutant. Further, in contrast to cells infected with wild-type serotype 2 adenovirus, dl331-infected cells contain increased eIF-2α kinase activity. These results indicate that VA RNA_I plays a role in suppressing eIF-2α kinase activity during adenovirus infection of HeLa cells.**

Copious amounts of two small, virus-associated (VA) RNAs are present in adenovirus-infected cells during the late phase of infection (1, 2). These RNAs are transcribed by RNA polymerase III from two genes located at about 30 map units on the adenovirus serotype 2 (Ad2) or adenovirus serotype 5 (Ad5) genome and are referred to as VA RNA_I and VA RNA_{II} (2–4). VA RNA_I is present in a 40-fold excess over VA RNA_{II} during late infection, and its presence is required for efficient translation of late mRNAs (5, 6). The role of VA RNA_I in translation has been studied largely through the use of the deletion mutant Ad5 dl331. This mutant lacks 29 base pairs within the VA RNA_I gene and fails to produce VA RNA_I (5). Cells infected with this mutant contain normal levels of viral mRNA but produce 1/10th to 1/8th as much protein at late times of infection. A second mutant, dl328, which produces normal amounts of VA RNA_I but no VA RNA_{II}, does not exhibit any translational block during late infection (5). Cell-free extracts prepared from HeLa cells infected with the wild-type virus (Ad2) or extracts from uninfected cells are capable of translating host and viral mRNAs, while similar extracts derived from cells infected with the dl331 mutant fail to translate host, viral, or heterologous mRNAs (7). The translational defect occurs at the level of polypeptide chain initiation (6, 7) and results from an inability of eukaryotic initiation factor 2 (eIF-2) to function catalytically (7).

During polypeptide chain initiation in eukaryotes (for reviews, see refs. 8 and 9), eIF-2 forms a ternary complex with GTP and the aminoacylated initiator tRNA, Met-$tRNA_i$. The ternary complex then binds to a 40S ribosomal subunit, forming a 40S initiation complex. Subsequent reactions lead to mRNA binding and the joining of the 60S ribosomal subunit to the 40S initiation complex to form an 80S initiation complex. During subunit joining, the ribosome-bound GTP is hydrolyzed and released together with eIF-2 as an eIF-2·GDP complex. To enter into an additional round of initiation, eIF-2-bound GDP must be replaced by GTP, since Met-$tRNA_i$ binds only to eIF-2·GTP. This reaction is catalyzed by an initiation factor called GEF, the guanine nucleotide exchange factor (9–13). GEF activity in reticulocytes is regulated by the phosphorylation state of eIF-2 (for a review see ref. 9). Two protein kinases are present within reticulocytes that phosphorylate the α subunit (38,000 M_r) of eIF-2. One of these kinases is referred to as the heme-controlled inhibitor of protein synthesis (HCI), and it is activated by heme deficiency, as well as by other stimuli, in reticulocytes. The second kinase is referred to as the double-stranded RNA-activated inhibitor of protein synthesis (DAI), and it is activated by very low concentrations of double-stranded RNA (ds RNA). Paradoxically, higher concentrations of ds RNA block the activation of this kinase. In cells other than reticulocytes, synthesis of DAI is induced by interferon. Phosphorylation of eIF-2 by either of these kinases leads to the subsequent formation of a catalytically inactive complex between GEF and the phosphorylated eIF-2 (14–17).

We show here that extracts of dl331-infected cells contain a protein kinase capable of phosphorylating eIF-2α and thereby blocking the activity of GEF and recycling of eIF-2.

MATERIALS AND METHODS

Assays. Conditions for all assays are given in the appropriate figure legends. Protein synthesis was measured as described by Reichel *et al.* (7). Ternary complex formation between eIF-2, GTP, and [^{3}H]Met-$tRNA_i$ was assayed according to Siekierka *et al.* (11). The inhibition by HCI of GEF-stimulated ternary complex formation and the phosphorylation of eIF-2 in the α subunit by HCI and [γ-^{32}P]ATP were as described (11). Protein was determined by the Bradford (18) procedure with bovine serum albumin as the standard.

Preparations. Growth of HeLa cells, infection with virus, and preparation of extracts were as in previous work (7). GEF was partially purified from cell extracts by chromatography on heparin-Sepharose 4B (19). Crude cell-free extracts

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Abbreviations: eIF-2, eukaryotic initiation factor 2; GEF, guanine nucleotide exchange factor; Met-$tRNA_i$, eukaryotic initiator methionyl-tRNA; HCI, heme-controlled inhibitor of protein synthesis (an eIF-2α kinase); eIF-2(αP), α-subunit-phosphorylated eIF-2; ds RNA, double-stranded RNA; DAI, ds RNA-activated inhibitor of protein synthesis (an eIF-2α kinase); VA RNA, virus-associated RNA; Ad2 and Ad5, wild-type adenovirus of serotypes 2 and 5; dl331, an adenovirus deletion mutant.

(1 ml, about 10 mg of protein) were applied to individual heparin-Sepharose 4B columns (0.9 × 1.5 cm) that were equilibrated in buffer containing 20 mM Tris·HCl at pH 7.5, 5 mM 2-mercaptoethanol, 0.2 mM EDTA, 10% (vol/vol) glycerol (buffer A), and 200 mM KCl. Columns were washed with this buffer until the absorbance at 280 nm was below 0.1. Protein was eluted with buffer A containing 500 mM KCl and dialyzed for 5 hr against buffer A containing 50 mM KCl. This preparation is referred to as HeLa GEF in the text. Further purification following essentially the same procedure used for rabbit reticulocyte eIF-2·GEF (19) yielded nearly homogeneous preparations of HeLa cell eIF-2·GEF (to be published). Partially purified rabbit reticulocyte eIF-2 (CM 350) was prepared according to de Haro and Ochoa (20). Highly purified reticulocyte eIF-2 (80–90% pure) was prepared according to the procedure of Benne *et al.* (21). HCI was prepared according to the procedure of Ranu and London (22).

RESULTS

An Inhibitor of Protein Synthesis in dl331-Infected Cells. Extracts of HeLa cells infected with the adenovirus mutant dl331 exhibit a translational defect (5). They fail to translate efficiently both endogenous and exogenous mRNAs as a result of a lesion at the level of polypeptide chain initiation (6, 7). In principle the defect could be due to the lack of an essential initiation factor or, alternatively, to the presence of an inhibitor that blocks its activity. To distinguish between these alternatives, we mixed the inactive dl331 extract with translationally active extracts from uninfected or wild-type Ad2-infected HeLa cells. If an inhibitor is present in the dl331 extract, it would be expected to suppress translation in the mixture. On the other hand, if the defect is the simple deficiency of a required component, the dl331 extract should be rescued in the mixed incubation. No such rescue was seen. On the contrary, the dl331 extract depressed protein synthesis below the level expected for the uninfected or Ad2-infected extract (shown by the dashed line in Fig. 1*A*), consistent with the presence of an inhibitor of translation. To confirm this inference, we assayed the HeLa cell extracts in the rabbit reticulocyte lysate, after first treating them with micrococcal nuclease to destroy their endogenous mRNA. The results (Fig. 1*B*) indicate that all the HeLa extracts were inhibitory at high concentration, but that the dl331 extract contains a much more potent inhibitor than either the uninfected or Ad2-infected extract. For example, when the HeLa extracts were present at 1/8th the level of the reticulocyte lysate, inhibitions of only 11% and 5% were observed with the uninfected and Ad2-infected cell extracts, respectively, compared to 50% with the dl331 extract. Thus, an inhibitor of protein synthesis accumulates in dl331-infected cells, and its nature is explored below.

Reduced GEF Activity in dl331 Extracts. In earlier work, we showed that the translational defect in dl331 extracts is overcome by the addition of either eIF-2 or GEF (7). GEF, free or complexed with eIF-2 (eIF-2·GEF), was much more efficient than eIF-2 in restoring translation in dl331 extracts (7). Since GEF allows eIF-2 to function catalytically, the ability of free GEF to restore translation in dl331 extracts suggests that a significant proportion of endogenous eIF-2 is not altered in these extracts. This is similar to what is observed in heme-deficient reticulocyte lysates. During heme deficiency, protein synthesis is inhibited by 90%, while only 20–30% of total cellular eIF-2 is α-phosphorylated (14, 24). Because α-phosphorylated eIF-2 forms a nondissociable complex with GEF, only low amounts of α-phosphorylated eIF-2 are required to sequester the limiting amount of GEF. To demonstrate directly that HeLa cell GEF activity is altered during infection by the adenovirus mutant dl331, we assayed partially purified GEF from various HeLa cell extracts for its ability to stimulate ternary complex formation from eIF-2·GDP (11). GEF was partially purified from uninfected HeLa cells and from cells that were infected with wild-type adenovirus (Ad2) or the adenovirus mutant dl331. Cell-free extracts were prepared and GEF was partially purified by chromatography on heparin-Sepharose 4B (*Materials and Methods*). As seen in Fig. 2, Ad2-infected HeLa cells exhibited GEF activity of the same magnitude as uninfected cells, whereas dl331-infected cells contained greatly reduced levels of GEF activity.

To show that the ternary complex stimulatory activity ob-

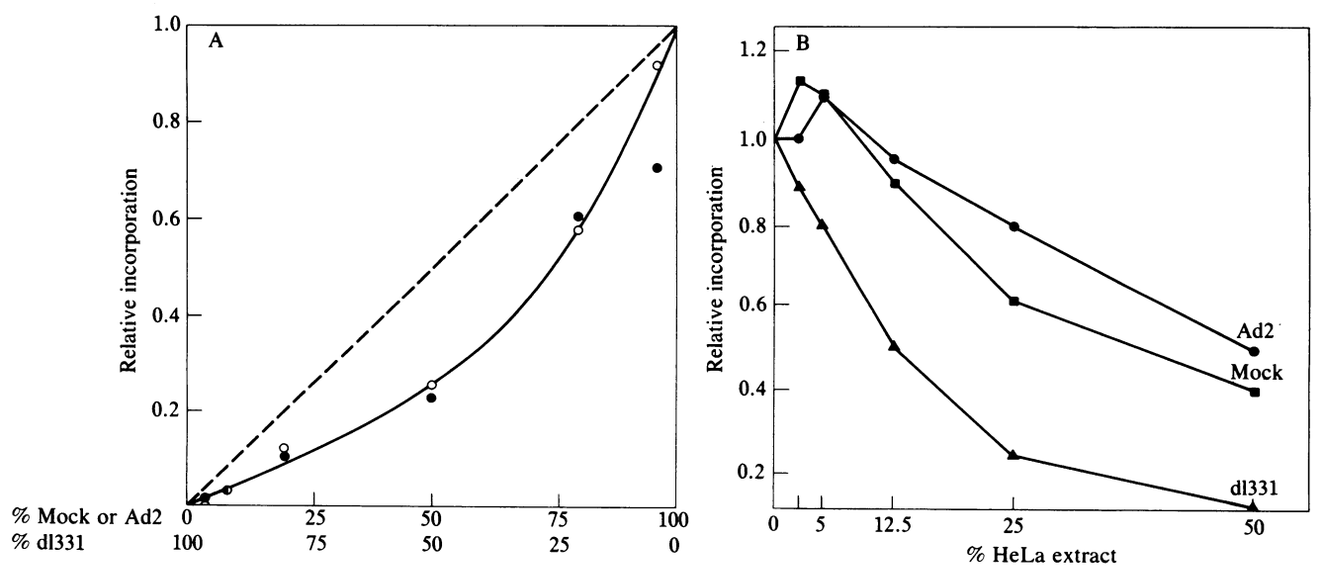

FIG. 1. Detection of a translational inhibitor in dl331 extracts. Protein synthesis was measured by incorporation of [^{35}S]methionine into protein. (*A*) Reaction mixtures contained a total of 12.5 μl of HeLa cell extract composed of various proportions of uninfected (mock) and dl331 extracts (●) or Ad2 and dl331 extracts (○). Maximal incorporation was 2.2 × 10^5 and 2.0 × 10^5 cpm per 5-μl sample, respectively. All HeLa cell extracts contained approximately 25 mg of protein per ml. (*B*) Incubation mixtures containing untreated rabbit reticulocyte lysate (5 μl per 12.5-μl reaction mixture) were supplemented with micrococcal nuclease-treated HeLa extracts (up to 2.5 μl per 12.5-μl mixture). A relative incorporation of 1.0 corresponds to 8.8 × 10^5 cpm per 5-μl sample.

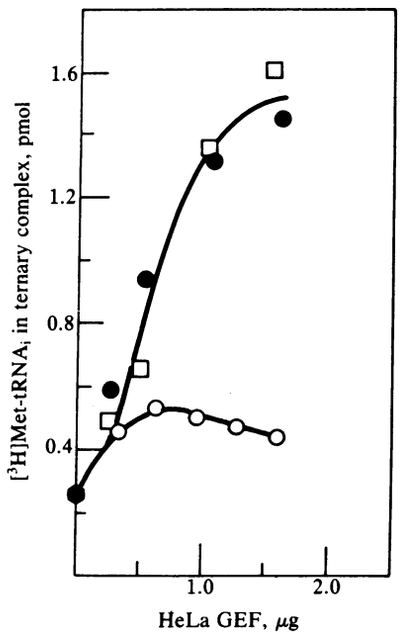

FIG. 2. Stimulation of ternary complex formation by partially purified GEF from Ad2 and dl331-infected HeLa cells. GEF was partially purified from HeLa cells that were uninfected or infected with either Ad2 or dl331 virus. GEF was assayed by its ability to stimulate the formation of a ternary complex between eIF-2, GTP, and Met-tRNA$_i$ (Met-tRNA$_i$·eIF-2·GTP) in the presence of Mg^{2+} and with eIF-2 present as eIF-2·GDP (11). Reaction mixtures (50 μl) contained 20 mM Tris·HCl at pH 7.5, 100 mM KCl, 2 mM 2-mercaptoethanol, 1 mM $Mg(OAc)_2$, 60 μM GTP (purified as in ref. 11), 10.2 pmol of [^{3}H]Met-tRNA$_i$ from yeast (51,000 cpm/pmol), 2.0 μg of CM 350 eIF-2·GDP (about 25% pure eIF-2), and increasing amounts of partially purified GEF. Reaction mixtures were incubated for 6 min at 30°C and filtered through nitrocellulose membranes, and retained radioactivity was measured as in previous work (11). ●, No GEF; □, uninfected HeLa cell GEF; ●, Ad2-infected cells; and ○, dl331-infected cells.

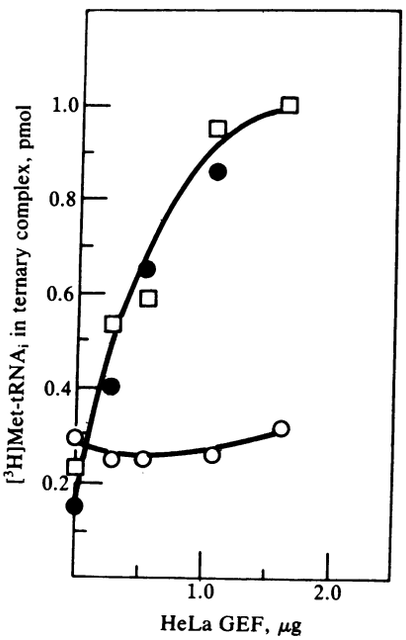

FIG. 3. Inhibition of partially purified HeLa cell GEF activity from Ad2-infected cells by phosphorylation of eIF-2 with HCI. The reaction was carried out in two steps, in which eIF-2 was phosphorylated in the first step and ternary complex stimulation by GEF was measured in the second step. Step I samples (20 μl) contained 20 mM Tris·HCl at pH 7.5, 2 mM $Mg(OAc)_2$, 2 mM 2-mercaptoethanol, 0.2 mM ATP, 1.9 μg of partially purified HCI, and 2 μg of CM 350 eIF-2·GDP. Controls lacking either ATP or HCI were also included. Samples were incubated for 6 min at 30°C, after which time they were supplemented with 100 mM KCl, 60 μM GTP, 10.2 pmol of [^{3}H]Met-tRNA$_i$ (51,000 cpm/pmol), and increasing amounts of Ad2-infected HeLa cell GEF. Samples (50 μl) were further incubated for 6 min at 30°C, after which time ternary complex was measured as in Fig. 1*A*. ○, Complete reaction mixture; □, ATP omitted in step I; ●, HCI omitted in step I.

served in uninfected and Ad2-infected cells is specifically due to GEF, we examined the sensitivity of the reaction to phosphorylation of eIF-2 by HCI. As mentioned previously, phosphorylation of the α subunit of eIF-2 by HCI results in the formation of a nondissociable complex between the phosphorylated eIF-2 and GEF [eIF-2(αP)·GEF] which is catalytically inactive. Since eIF-2 is the only known substrate for HCI and guanine nucleotide exchange is the only reaction known to be affected by α subunit phosphorylation of eIF-2, the experiment is diagnostic for GEF. As shown in Fig. 3, phoshorylation of eIF-2 by HCI and ATP resulted in the complete inhibition of the stimulation of ternary complex formation by GEF from Ad2-infected HeLa cells. Controls lacking either HCI or ATP showed no inhibition, demonstrating that the activity is due to GEF.

These findings show that the activity of GEF in dl331-infected HeLa cells is greatly reduced as compared to either uninfected or Ad2-infected cells. The results also demonstrate that HeLa cell GEF activity is specifically inhibited by α-phosphorylated eIF-2, as is the case for rabbit reticulocyte GEF, suggesting that phosphorylation plays a role in establishing the translational block observed in dl331-infected HeLa cells.

dl331 Extracts Contain a Protein Kinase Activity That Phosphorylates the α Subunit of eIF-2. The results presented in the two previous sections prompted us to compare extracts derived from Ad2- and dl331-infected HeLa cells for their ability to phosphorylate the α subunit of eIF-2. We reasoned that, in dl331-infected HeLa cells, GEF may be inactivated because of increased eIF-2α kinase activity, which would raise the concentration of α-phosphorylated eIF-2 in these cells. To assay phosphorylation of eIF-2, we incubated highly purified rabbit reticulocyte eIF-2 with [γ-^{32}P]ATP, Mg^{2+}, and increasing amounts of either Ad2 or dl331 extract. The reaction products were examined by sodium dodecyl sulfate/polyacrylamide gel electrophoresis and autoradiography. As shown in Fig. 4, the dl331 extract contains an active protein kinase that phosphorylates the α subunit of eIF-2. Very little phosphorylation of the α subunit was observed with the Ad2 extract. Likewise, little activity was observed in extracts of uninfected cells (not shown).

Since the steady-state level of α-phosphorylated eIF-2 is determined by the relative rates of phosphorylation and dephosphorylation, we also examined the rate of dephosphorylation of exogenously added eIF-2(α^{32}P) by Ad2 and dl331 extracts. As shown in Fig. 5, the rates of dephosphorylation of eIF-2(α^{32}P) by phosphatase(s) present in the extracts were similar for Ad2- and dl331-infected cells. Thus, the ability of dl331 extracts to phosphorylate eIF-2 is due to the activation of a protein kinase rather than to the inability of dl331 extracts to dephosphorylate eIF-2(α^{32}P).

We also examined the ability of dl331 extracts to inhibit GEF-stimulated ternary complex formation in an ATP-dependent manner. The two-step assay protocol was similar to that used previously to demonstrate the inhibition of rabbit reticulocyte and HeLa cell GEF activities by eIF-2 phosphorylated with HCI (ref. 11 and Fig. 3). In the first step, eIF-2 was incubated with ATP, Mg^{2+}, and various amounts of Ad2 or dl331 extract to permit phosphorylation of the α subunit of eIF-2. In the second step, the remaining compo-

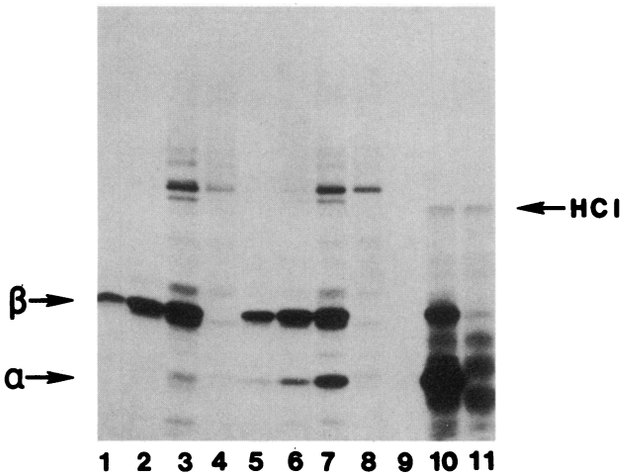

FIG. 4. Phosphorylation of the α subunit of eIF-2 upon incubation with dl331 cell-free extracts. Cell-free extracts were prepared from Ad2- and dl331-infected HeLa cells; in these experiments, to remove endogenous ATP, cell-free extracts were extensively dialyzed against buffer containing 20 mM Tris·HCl at pH 7.5, 50 mM KCl, 5 mM 2-mercaptoethanol, 0.2 mM EDTA, and 5% glycerol. Reaction mixtures (30 μl) contained 20 mM Tris·HCl at pH 7.5, 2 mM 2-mercaptoethanol, 4 mM $Mg(OAc)_2$, 1 mM [γ-^{32}P]ATP (1000 cpm/pmol), 3 μg of highly purified reticulocyte eIF-2, and amounts of extract indicated. Reaction mixtures were incubated for 6 min at 30°C and subjected to sodium dodecyl sulfate/polyacrylamide gel electrophoresis and autoradiography as described (19). Lanes 1–3, complete reaction with 0.25, 1.0, and 4.0 μg of Ad2 extract, respectively; lane 4, reaction with 4 μg of Ad2 extract and no eIF-2; lanes 5–7, complete reaction with 0.25, 1.0, and 4.0 μg of dl331 extract, respectively; lane 8, reaction with 4.0 μg of dl331 extract and no eIF-2; lane 9, reaction with no extract; lane 10, complete reaction with HCI added instead of extracts; and lane 11, reaction with HCI alone and no eIF-2. Arrows indicate the position of the α subunit and β subunit of eIF-2 and the autokinase band of HCI.

nents for ternary complex formation were added together with partially purified GEF from uninfected HeLa cells, and the mixture was further incubated. As shown in Fig. 6, preincubation of eIF-2 with dl331 extract and ATP resulted in the inhibition of GEF-dependent ternary complex formation. Excluding ATP from the first step greatly reduced the extent of the inhibition. When assayed under identical conditions, the Ad2 extract was much less inhibitory. Similar data were obtained when rabbit reticulocyte GEF was used instead of HeLa GEF in the second step (data not shown). These results strongly indicate that the translational block observed in cell-free extracts from dl331-infected HeLa cells is due to the activation of a kinase(s) that phosphorylates the α subunit of eIF-2. The resulting increase in α-phosphorylated eIF-2 can directly inhibit GEF by forming a catalytically inactive complex, eIF-2(αP)·GEF.

DISCUSSION

The adenovirus mutant Ad5 dl331 fails to produce VA RNA_i, and HeLa cells infected with this mutant fail to translate mRNAs efficiently at late times in infection (5–7). Using cell-free extracts from dl331-infected HeLa cells, which exhibit a greatly reduced level of protein synthesis, we showed that normal translational activity can be restored by the addition of eIF-2, eIF-2·GEF, or free GEF (7). GEF (as the eIF-2·GEF complex) was 15 times more effective than eIF-2 alone in restoring translation in dl331 cell-free extracts (7). Our present investigation shows that extracts derived from dl331-infected HeLa cells have very little GEF activity as compared to extracts derived from uninfected or Ad2-infected HeLa cells. Addition of exogenous reticulocyte GEF or

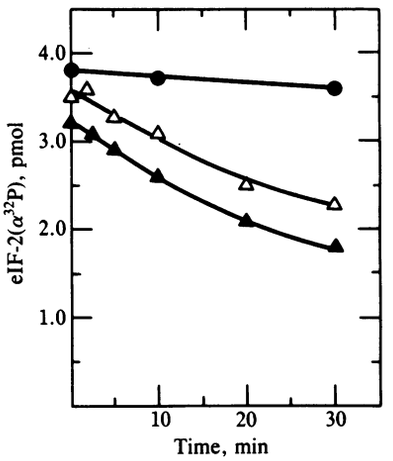

FIG. 5. Dephosphorylation of α-phosphorylated eIF-2 by Ad2 and dl331 cell-free extracts. Highly purified eIF-2 was phosphorylated in the α subunit as described (11). Reaction mixtures (50 μl) contained 20 mM Tris·HCl at pH 7.5, 2 mM 2-mercaptoethanol, 85 mM KCl, 1 mM $Mg(OAc)_2$, 0.51 μg of eIF-2(α^{32}P) (3300 cpm/pmol), and 30 μg of either Ad2 or dl331 dialyzed extract. Control samples without extract were also included. Reaction mixtures were incubated at 30°C for the times indicated and subjected to sodium dodecyl sulfate/polyacrylamide gel electrophoresis (19). After electrophoresis, the bands corresponding to the α subunit of eIF-2 were excised and placed in scintillation vials containing 0.8 ml of 30% (wt/vol) H_2O_2. The gel was dissolved by heating at 75°C with shaking, after which time 10 ml of scintillation fluid (Hydrofluor, National Diagnostics) was added and radioactivities of samples were measured in a Beckman LS-100 scintillation counter. △, eIF-2(α^{32}P) and Ad2 extract; ▲, eIF-2(α^{32}P) and dl331 extract; and ●, eIF-2(α^{32}P) without extract.

eIF-2·GEF to dl331 extracts would therefore be expected to restore translation by rectifying this deficit. In an analogous manner, addition of exogenous reticulocyte eIF-2·GEF to heme-deficient reticulocyte lysates restores translation to normal levels (19). Similarly, eIF-2, at levels significantly higher than eIF-2·GEF, can also restore translation in heme-deficient reticulocyte lysates. In the latter case, eIF-2 functions stoichiometrically (one mole of eIF-2 promoting the synthesis of one mole of globin), whereas, in the former case, eIF-2·GEF functions catalytically (14). It is very likely that the greater efficiency of eIF-2·GEF in restoring transla-

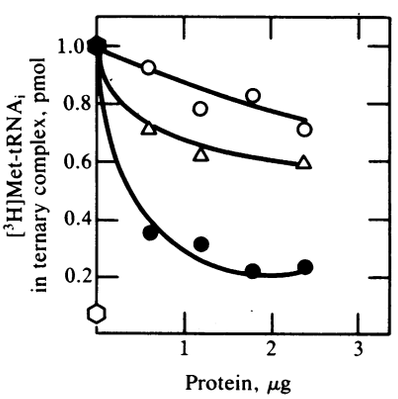

FIG. 6. ATP-dependent inhibition of HeLa cell GEF by dl331 cell-free extracts. Conditions were as described in the legend to Fig. 3 with the following modifications: Step I volume was 30 μl and the ATP concentration was 1 mM. Amounts of dialyzed Ad2 and dl331 extract are indicated. In step II, the reaction volume was 50 μl and 1 μg of partially purified GEF from uninfected HeLa cells was used. ○, Ad2 extract with ATP; ●, dl331 extract with ATP; △, dl331 extract without ATP; ○, no extract, no HeLa GEF; ●, no extract, plus HeLa GEF.

tion in dl331 cell-free extracts is due to its ability to act catalytically.

Complex formation with α-phosphorylated eIF-2 [eIF-2(αP)·GEF] is the only mechanism known whereby GEF activity is regulated. Cell-free extracts from dl331-infected HeLa cells contain an eIF-2α kinase activity that is barely detectable in extracts from Ad2-infected cells. The activation of this kinase in dl331-infected cells would increase the level of eIF-2(αP), which in turn would inactivate GEF by the formation of an eIF-2(αP)·GEF complex. It appears that VA RNA_I plays a role in antagonizing this activity by means that are not yet clear. In theory, this could be accomplished by preventing the synthesis or activation of the kinase or by blocking its phosphorylation activity. A further possibility, that VA RNA activates a phosphatase that dephosphorylates eIF2(αP), is not supported by our data. It is now clear, however, that the increased levels of α-phosphorylated eIF-2 in dl331-infected HeLa cells can account for the reduced GEF activity, making it seem rather unlikely that VA RNA acts directly on any of the components of the initiation pathway.

The exact nature of the eIF-2α kinase(s) remains to be established. Most probably it is a host cell activity, although a possible viral origin cannot be excluded at present. In considering potential candidates for the kinase, a plausible case can be made that DAI is responsible for the observed phosphorylation since the activity is modulated by VA RNA_I. As noted previously (7), VA RNA_I can form stable double-stranded RNA structures either with itself or with other cellular RNAs. Considering the high concentrations of VA RNA_I found in HeLa cells during Ad2 infection, one may expect to find rather high concentrations of RNA duplex. High concentrations of ds RNA are known to block the activation of DAI (23) and may do so in Ad2-infected HeLa cells. The lack of VA RNA_I in dl331-infected cells would permit the activation of DAI and lead to phosphorylation of the α subunit of eIF-2. A definitive answer as to the role of VA RNA must await purification and characterization of the kinase(s) involved.

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1. Reich, P. R., Rose, J., Forget, B. & Weissman, S. M. (1966) *J. Mol. Biol.* **17,** 428–439.
2. Söderlung, H., Pettersson, U., Vennstrom, B., Philipson, L. & Mathews, M. B. (1976) *Cell* **7,** 585–593.
3. Mathews, M. B. (1975) *Cell* **6,** 223–229.
4. Pettersson, U. & Philipson, L. (1975) *Cell* **6,** 1–4.
5. Thimmappaya, B., Weinberger, C., Schneider, R. J. & Shenk, T. (1982) *Cell* **31,** 543–551.
6. Schneider, R. J., Weinberger, C. & Shenk, T. (1984) *Cell* **37,** 291–298.
7. Reichel, P. A., Merrick, W. C., Siekierka, J. & Mathews, M. B. (1985) *Nature (London)* **313,** 196–200.
8. Jagus, R., Anderson, W. F. & Safer, B. (1981) *Prog. Nucleic Acid Res. Mol. Biol.* **25,** 127–185.
9. Ochoa, S. (1983) *Arch. Biochem. Biophys.* **223,** 325–349.
10. Clemens, M. J., Pain, V. M., Wong, S.-T. & Henshaw, E. C. (1982) *Nature (London)* **296,** 93–95.
11. Siekierka, J., Mauser, L. & Ochoa, S. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 2537–2540.
12. Konieczny, A. & Safer, B. (1983) *J. Biol. Chem.* **258,** 3402–3408.
13. Panniers, R. & Henshaw, E. C. (1983) *J. Biol. Chem.* **258,** 7928–7934.
14. Safer, F., Jagus, R., Konieczny, A. & Crouch, D. (1982) in *Interaction of Translational and Transcriptional Control in the Regulation of Gene Expression*, eds. Grunberg-Manago, M. & Safer, B. (Elsevier, New York), Vol. 24, pp. 311–325.
15. Matts, R. L., Levin, D. H. & London, I. M. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 2559–2563.
16. Siekierka, J., Veeraswamy, M. & Ochoa, S. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 352–356.
17. Goss, D. J., Parkhurst, L. J., Mehta, H. B., Woodley, C. L. & Wahba, A. J. (1984) *J. Biol. Chem.* **259,** 7374–7377.
18. Bradford, M. M. (1976) *Anal. Biochem.* **72,** 248–254.
19. Siekierka, J., Mitsui, K.-I. & Ochoa, S. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 220–223.
20. de Haro, C. & Ochoa, S. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 1741–1745.
21. Benne, R., Wong, C., Luedi, M. & Hershey, J. W. B. (1975) *J. Biol. Chem.* **251,** 7675–7681.
22. Ranu, R. S. & London, I. M. (1979) *Methods Enzymol.* **60,** 459–484.
23. Hunter, T., Hunt, T., Jackson, R. J. & Robertson, H. D. (1975) *J. Biol. Chem.* **250,** 409–417.
24. Leroux, A. & London, I. M. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 2147–2151.