MOLECULAR AND CELLULAR BIOLOGY, Dec. 1986, p. 4493–4498
0270-7306/86/124494-06$02.00/0

Vol. 6, No. 12

An Adenovirus Mutant Unable To Express VAI RNA Displays Different Growth Responses and Sensitivity to Interferon in Various Host Cell Lines

JAN KITAJEWSKI,[1] ROBERT J. SCHNEIDER,[1]† BRIAN SAFER,[2] AND THOMAS SHENK[1]*

Department of Molecular Biology, Princeton University, Princeton, New Jersey 08544,[1] and Section on Protein Biosynthesis, Laboratory of Molecular Hematology, National Heart, Lung and Blood Institute, Bethesda, Maryland 20205[2]

Received 13 April 1986/Accepted 29 August 1986

The VAI RNA of adenovirus is a small, RNA polymerase III-transcribed species required for the efficient translation of host cell and viral mRNAs late after infection. VAI RNA prevented activation of the interferon-induced P1/eIF-2α kinase. In its absence the kinase was activated, eIF-2α was phosphorylated, and translational initiation was inhibited. H5*dl*331 (*dl*331), a mutant which cannot express VAI RNA, grew poorly in 293 cells but generated wild-type yields in KB cells. The growth phenotype of the mutant appeared to correlate with the kinetics of kinase induction and activation. Active kinase appeared more rapidly in cell extracts prepared from infected 293 cells, in which *dl*331 grew poorly, than in extracts of KB cells, in which the mutant grew well. However, when kinase was induced in KB cells by interferon treatment and then activated subsequent to *dl*331 infection, viral protein synthesis was less severely inhibited than in interferon-treated 293 cells. Thus, activated kinase per se is insufficient to severely inhibit *dl*331 protein synthesis in KB cells.

The adenovirus VA RNAs are small transcripts (160 nucleotides) synthesized in large amounts late after infection by RNA polymerase III (13, 24, 28). There are two VA RNA genes, designated VAI and VAII, located at about 30 map units on the adenovirus type 5 (Ad5) chromosome (10, 12). To explore the function of these RNAs, we constructed two Ad5 variants, each of which fails to encode one of the VA species (26). The mutant that fails to produce the VAI RNA (*dl*331) grows more poorly than its parent in the human 293 cell line. Analysis of its defect indicated that VAI RNA is required for the efficient initiation of translation late after infection (22, 26). Both viral and host cell protein synthesis become dependent on VAI RNA late after infection (14, 26).

The translational defect results from activation of the P1/eIF-2α kinase and subsequent loss of initiation factor 2 (eIF-2) activity (14, 21, 23). The role of the kinase in loss of eIF-2 activity has been documented in several systems (for a review, see reference 6). During the initiation process, eIF-2, which is composed of three subunits (α, β, γ), functions in a ternary complex with GTP and Met-tRNA$_i$. GTP is hydrolyzed to GDP as the 80S initiation complex is formed and eIF-2 is released. The eIF-2-associated GDP must be exchanged for GTP before the factor can mediate a new round of initiation. This exchange is catalyzed by the GTP-recycling factor termed eIF-2B. It does not occur when the eIF-2α subunit has been phosphorylated. Rather, phosphorylated eIF-2 sequesters the limited quantities of eIF-2B in a tight complex, preventing the recycling reaction (for a review, see reference 16). Thus, GTP is no longer recycled, eIF-2 does not function catalytically, and the frequency of initiation is decreased.

Synthesis of the P1/eIF-2α kinase is induced by interferon, and it is activated by double-stranded RNA (dsRNA) (9, 15, 18, 29). VAI RNA prevents activation but not induction of the kinase in response to interferon (8). Furthermore, VAI RNA can inhibit activation of the kinase by dsRNA in extracts prepared from interferon-treated cells (8, 11). Not surprisingly, whereas wild-type Ad5 is insensitive to interferon, growth of *dl*331 in 293 cells is inhibited by the antiviral agent (8).

It makes good sense that the kinase is activated in extracts prepared from *dl*331-infected cells that have been pretreated with interferon. The kinase is induced by interferon and subsequently activated since VAI RNA is not available to inhibit the process. However, activated kinase was originally observed in *dl*331-infected 293 cells that were not treated with interferon (21, 23). In this instance the agent responsible for induction of the kinase is unknown.

In contrast to its growth response in 293 cells, we report here that *dl*331 grows as well as wild-type virus in KB cells. The growth phenotype of the mutant appears to correlate with the kinetics of P1/eIF-2α kinase induction and activation. Active kinase appears more rapidly in cell extracts prepared from 293 cells, in which *dl*331 grows poorly, than in extracts of KB cells, in which it grows well. Kinase is efficiently induced in both cell types by interferon treatment and is activated subsequently to *dl*331 infection. Although *dl*331-directed late translation is dramatically reduced in interferon-treated 293 cells, mutant translation is only modestly affected in KB cells. Thus, activated kinase is insufficient to severely inhibit protein synthesis in *dl*331-infected KB cells.

MATERIALS AND METHODS

Viruses and cells. Wild-type Ad5 (H5*wt*300) is a plaque-purified derivative of a stock originally obtained from H. Ginsberg, Columbia University (New York, N.Y.). H5*dl*309 (*dl*309) is a phenotypically wild-type derivative of H5*wt*300 that carries a series of altered restriction endonuclease cleavage sites (7). *dl*331 lacks 29 base pairs within the

* Corresponding author.
† Present address: Department of Biochemistry, New York University Medical Center, New York, NY 10016.

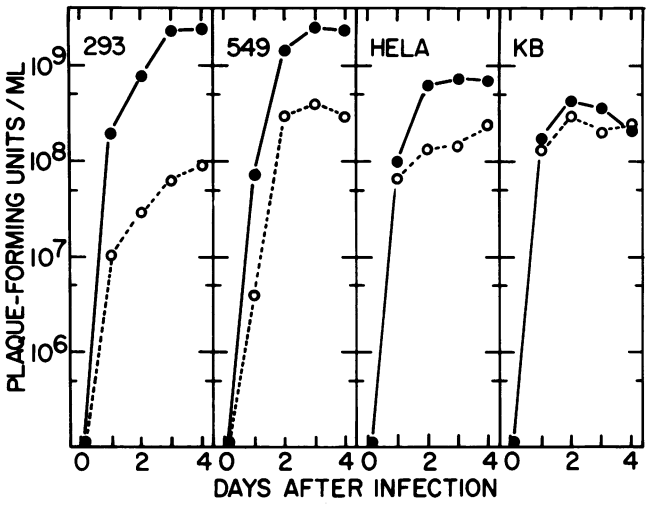

FIG. 1. Growth kinetics of mutant and wild-type viruses on a variety of human cell lines. Cells were infected at a multiplicity of 3 PFU per cell, and virus yield was measured by plaque assay on 293 cells. Symbols: ●, *dl*309, ○, *dl*331.

intragenic control region of the VAI RNA gene and, as a result, fails to produce VAI RNA (26). All infections were at a multiplicity of 20 PFU per cell unless otherwise noted in figure legends.

The 293 cell line (a human embryonic kidney cell line transformed with a DNA fragment carrying the left 11% of the Ad5 genome) has been described previously (3). HeLa and KB cell lines were obtained from H. Young, Columbia University, and A549 cells were obtained from the American Type Culture Collection (Rockville, Md.). All cells were propagated in medium containing 10% calf serum. Plaque assays were performed on 293 cells as described previously (26). For interferon treatment 1,000 U of human αA interferon per ml was used, which was kindly provided by S. Pestka, Roche Institute (Nutley, N.J.). Cell cultures were pretreated with interferon for 24 h prior to infection and were then refed with medium containing interferon after infection.

RNA preparation and analysis. Procedures for isolation of cytoplasmic poly(A)$^+$ RNA from infected cells and for Northern-type RNA blot analysis have been described previously (4). The DNA probe used to analyze L5 mRNAs was a recombinant plasmid which carried the Ad5 DNA segment between 88.3 and 91 map units. To monitor VA RNA synthesis, infected cell nuclei were isolated and labeled for 30 min with [α-^{32}P]UTP (500 μCi/ml, 600 Ci/mmol) before total nuclear RNA was prepared and analyzed by electrophoresis (26).

Polypeptide analysis. To analyze polypeptide synthesis, infected cells were labeled with [^{35}S]methionine (50 μCi/ml, 1,100 Ci/mmol) for 60 min. Preparation of cytoplasmic extracts, immunoprecipitations, and sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis were carried out as described previously (20). Fiber immunoprecipitations used a polyclonal antibody prepared against electrophoretically purified fiber polypeptide as antigen. The fiber antibody was kindly provided by C. Anderson, Brookhaven National Laboratory.

Polypeptide phosphorylation. Extracts were prepared from cells 22 h after infection at a multiplicity of 20 PFU per ml. In vitro phosphorylation assays have been described in detail (17). Briefly, the reaction mixture contained 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; pH 7.5), 48 mM KCl, 2 mM dithiothreitol, 4 mM magnesium acetate, 100 mM ATP containing 10 μCi [γ-^{32}P]ATP (500 μCi/ml, 7,000 Ci/mmol), and 10 μl of S-10 extract containing about 20 μg of protein. Purified eIF-2 was included at 20 μg/ml. Samples were processed for electrophoresis after incubation at 30°C for 10 min. For immunoprecipitations sheep polyclonal antiserum raised against purified eIF-2 was used.

Phosphatase assay. Purified eIF-2 was phosphorylated with partially purified P1/eIF-2α kinase and [γ-^{32}P]ATP and then separated from residual labeled ATP by DEAE-cellulose chromatography and gel filtration on Sephadex G25. To assay for phosphatase, the ^{32}P-labeled eIF-2 was incubated at 30°C with 20 μg of S-10 extract in a final volume of 50 μl under the conditions described above for kinase assays, except that the [γ-^{32}P]ATP was omitted. After various times of incubation, fractions were removed, boiled in SDS-containing electrophoresis sample buffer, and subjected to electrophoresis in a 12.5% SDS-containing polyacrylamide gel.

RESULTS

Variable growth response for *dl*331 in different cell lines. A variety of human cell lines were tested for their ability to support the growth of *dl*331 (Fig. 1). Whereas the VAI mutant generated a 25-fold reduced yield compared with the wild-type virus in 293 cells, the two viruses displayed identical growth kinetics in KB cells. Intermediate growth responses for *dl*331 were observed in 549 and HeLa cells. The different growth responses were also evident when late polypeptide synthesis was monitored (Fig. 2). The 293 cells infected with wild-type virus (*dl*309) displayed a high rate of viral polypeptide synthesis by 20 h after infection, while *dl*331-infected 293 cells did not. Similar quantities of late viral polypeptides were produced in *dl*309- and *dl*331-infected KB cells. It is interesting that *dl*331 directed synthesis of near normal levels of late polypeptides in the HeLa

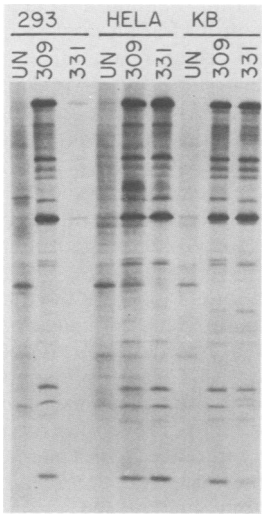

FIG. 2. Electrophoretic analysis of polypeptides synthesized in various cell lines late after infection with mutant or wild-type viruses. Cells were labeled with [^{35}S]methionine (50 μCi/ml) for 60 min 22 h after infection with *dl*309 (309) or *dl*331 (331) at a multiplicity of 20 PFU per cell (UN, uninfected cells). Extracts were prepared and subjected to electrophoresis in a 12.5% polyacrylamide gel containing 0.1% SDS.

cell line studied here, but we have previously observed a severe reduction in late protein synthesis in a different HeLa cell line (22).

Variable growth correlates with activation of P1/eIF-2α kinase. The basis for the differential growth response of *dl*331 in various cell lines was explored. Mutant *dl*331 continued to encode VAII RNA. In fact, it produces more of this RNA than wild-type virus (26). Since it probably functions similarly to the VAI species, it seemed possible that VAII RNA could be expressed at higher levels in the permissive KB cells than in 293 cells. However, this proved not to be the case (Fig. 3A). The same amount of VAII RNA was produced in each cell line tested. Similarly, *dl*331-encoded late mRNAs were not overproduced in the permissive KB cells (Fig. 3B).

Next, extracts were produced to test for activaion of the P1/eIF-2α kinase (Fig. 4). Both eIF-2α and eIF-2β subunits were phosphorylated in this experiment. The two subunits are phosphorylated by different kinases, and β-subunit phosphorylation is not known to affect eIF-2 activity (25, 27). As reported previously (21, 23), P1/eIF-2α kinase activity was substantially elevated by 20 h after infection of 293 cells with *dl*331. No increase over the uninfected cell level was observed in wild-type virus (*dl*309)-infected 293 cells. In contrast, no increase in kinase activity was observed in extracts prepared from *dl*331-infected KB cells 20 h after infection. To test whether kinase was induced but not activated in the 20-h KB cell extracts, assays were repeated in the presence of an activator (reovirus dsRNA). No increase in activity was detected under these conditions (data not shown), indicating that kinase was neither induced nor activated by 20 h after infection of KB cells with *dl*331. Increased kinase activity in the absence of added dsRNA was observed, however, in KB cell extracts prepared 32 or 44 h after infection with *dl*331 (Fig. 4). Thus, increased kinase activity can be observed in *dl*331-infected KB cells, but only after a delay relative to the 293 cell.

The apparent delay in production of active kinase in

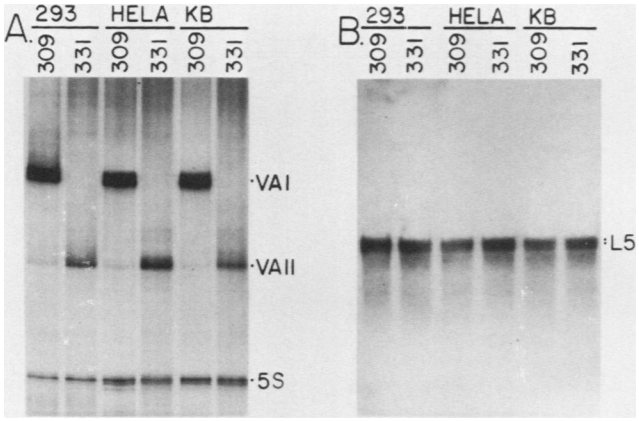

FIG. 3. Electrophoretic analysis of VA RNAs and L5-specific mRNAs synthesized in various cell lines after infection with mutant or wild-type viruses. 309, mutant *dl*309; 331, mutant *dl*331. (A). Analysis of VA RNAs. Nuclei were prepared 22 h after infection at a multiplicity of 20 PFU per cell and labeled for 30 min with [α-^{32}P]UTP (200 μCi/ml). Total nuclear RNA was prepared and subjected to electrophoresis in an 8% polyacrylamide gel containing 8 M urea (B). RNA blot analysis of L5-specific mRNAs. Cytoplasmic poly(A)$^+$ RNA was prepared 22 h after infection at a multiplicity of 20 PFU per cell, and 0.5-μg fractions were subjected to RNA blot analysis.

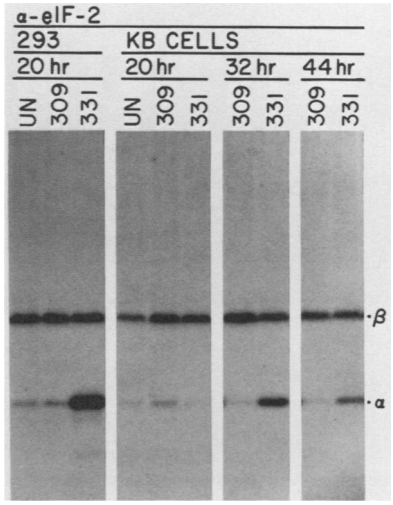

FIG. 4. Electrophoretic analysis of eIF-2 phosphorylated in extracts prepared from mutant or wild-type virus-infected 293 or KB cells. Extracts were prepared from uninfected cells (UN) or 22 h after infection with *dl*309 (309) or *dl*331 (331) at a multiplicity of 20 PFU per cell. Kinase assays were performed with added eIF-2 (20 μg/ml). After incubation in the presence of [γ-^{32}P]ATP for 10 min at 30°C, phosphorylated eIF-2 was immunoprecipitated and subjected to electrophoresis in a 12.5% polyacrylamide gel containing 0.1% SDS. eIF-2α and -β subunits are labeled.

*dl*331-infected KB cells could be an indirect effect of enhanced phsophatase activity that catalyzes the dephosphorylation of eIF-2α-P (1). To test phosphatase levels, phosphorylated eIF-2α was added to 293 or KB cell extracts, and its rate of dephosphorylation was monitored (Fig. 5). Dephosphorylation proceeded at the same rate in all extracts tested (50% in about 60 min), indicating that the phosphatase is present at the same level in 293 and KB cells and that its activity is indifferent to viral infection.

We conclude that the lag in production of active kinase in *dl*331-infected KB as compared with 293 cells is due at least in part to a delay in the induction and activation of the kinase in KB cells. This delay likely gives *dl*331 opportunity to synthesize its late polypeptides and generate normal yields in KB cells.

Growth response of *dl*331 is not likely owing to differential

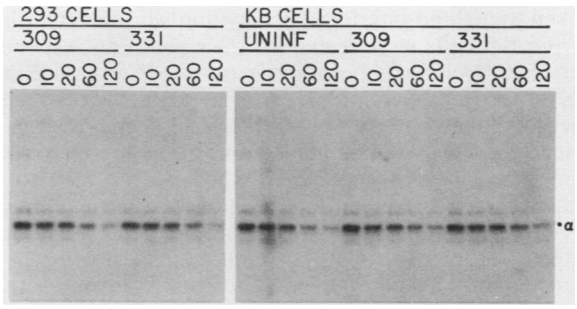

FIG. 5. Electrophoretic analysis of phosphorylated eIF-2 subjected to phosphatase activity present in extracts of mutant or wild-type virus-infected 293 or KB cells. Extracts were prepared from uninfected cells (UNINF) or 22 h after infection with *dl*309 (309) or *dl*331 (331). ^{32}P-labeled eIF-2 was added to the extracts and incubated at 30°C. After various times (indicated in minutes above the lanes), fractions were removed and subjected to electrophoresis in a 12.5% polyacrylamide gel containing 0.1% SDS.

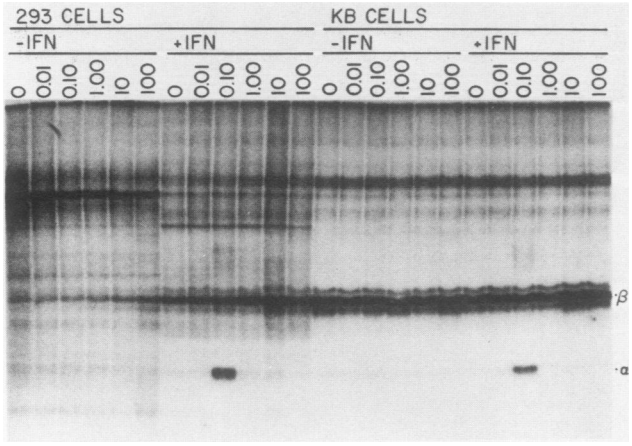

FIG. 6. Electrophoretic analysis of polypeptides phosphorylated in extracts prepared from interferon-treated (+IFN) or untreated (−IFN) 293 or KB cells. Extracts were prepared from either untreated cells or 24 h after the addition of 1,000 U of human αA interferon per ml. Kinase assays were performed in the presence of various quantities of added reovirus dsRNA (0 to 100 μg/ml; the amount is indicated over each lane). After incubation in the presence of [γ-^{32}P]ATP for 10 min at 30°C, all of the extracts were subjected to electrophoresis in a 12.5% polyacrylamide gel containing 0.1% SDS. eIF-2α and -β subunits are labeled.

production of interferon by 293 and KB cells. The lag in kinase expression in *dl*331-infected KB cells could result from differential production of interferon in KB as compared with 293 cells. If 293 cells produced interferon either very quickly after infection or in high amounts, it could feed back on the infected population to induce expression of the kinase. Accordingly, interferon production after infection of KB or 293 cells with either *dl*309 or *dl*331 was monitored by a vesicular stomatitis virus (VSV) plaque reduction assay on WISH cells. In this assay, WISH cells were treated with medium from the Ad5-infected cultures prior to infection with VSV. VSV is highly sensitive to interferon, and its ability to generate plaques is prevented in its presence. Although a 50% reduction in VSV plaquing ability could be observed in control experiments by pretreatment of WISH cells with 1 U of human αA interferon per ml, no reduction was observed by pretreatment with medium from infected KB or 293 cells (data not shown). Thus, neither cell line produces detectable extracellular interferon.

Even though no interferon production was detected, both 293 and KB cells were tested for their ability to respond to interferon by inducing synthesis of the P1/eIF-2α kinase (Fig. 6). Extracts were prepared from uninfected cells which either never saw interferon or which had been grown in the presence of 1,000 U of human αA interferon per ml for 20 h. Various amounts of reovirus dsRNA were added to the extracts to activate latent kinase, and the transfer of radioactive phosphate to the eIF-2α subunit was monitored. Neither cell line expressed substantial kinase activity in the absence of interferon treatment, and both expressed activity after treatment. Furthermore, kinase activity was induced in both cell lines by treatment with as little as 25 U of αA interferon per ml (data not shown). Thus, the P1/eIF-2α kinase was induced in both cells by levels of interferon which could be detected easily in the VSV plaque reduction assay.

One might still argue that low levels of cell-associated interferon are produced by the infected cell cultures that cannot be detected by assaying the culture medium. This condition could occur if the amount of interferon produced was submolar relative to cellular interferon receptors. To address this possibility, *dl*331-infected 293 cells were maintained in medium containing sufficient antibody to neutralize 250 U of β interferon per ml. Inclusion of antibody to β interferon had no effect on the appearance of kinase activity (data not shown). Thus, it appears very unlikely that extracellular interferon plays a role in the induction or activation of the P1/eIF-2α kinase in *dl*331-infected cells.

Polypeptide synthesis and yield do not correlate with kinase levels in *dl*331-infected KB cells treated with interferon. When KB or 293 cells were pretreated with interferon, no increase in P1/eIF-2α kinase activity was evident by 20 h after infection with wild-type virus (*dl*309) (Fig. 7). Latent kinase is induced but not activated in both cell types (8; data not shown). By 20 h after infection with *dl*331, kinase was activated to similar levels in both 293 and KB cells which were pretreated with interferon (Fig. 7). Curiously, late viral polypeptide synthesis was reduced to a lesser extent in interferon-pretreated KB than 293 cells. This is evident when either total infected cell proteins or immunoprecipitated fiber polypeptide was monitored (Fig. 8). Consistent with this observation, the yield of *dl*331 was nearly insensitive to interferon in KB cells, while it was reduced in 293 cells (Table 1). Thus, it appears that expression of activated kinase is not in and of itself sufficient to substantially interfere with *dl*331 growth.

DISCUSSION

The growth response of *dl*331 varies among different human cell lines (Fig. 1 and 2). Extremes are observed in 293 cells, in which the yield of *dl*331 is reduced about 25-fold, and KB cells, in which *dl*331 and wild-type (*dl*309) viruses generate similar yields. A key difference between 293 and KB cells appears to be the rapidity with which the P1/eIF-2α kinase is induced and activated subsequent to *dl*331 infection. Analysis of kinase activity in cell extracts indicates that its expression is delayed in KB as compared with 293 cells

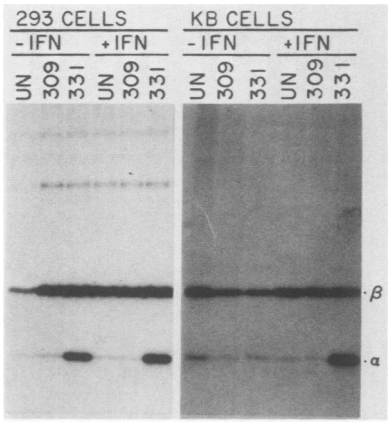

FIG. 7. Electrophoretic analysis of eIF-2 phosphorylated in extracts prepared from interferon-treated (+IFN) or untreated (−IFN) cells infected with mutant or wild-type viruses. Extracts were prepared from uninfected (UN) 293 or KB cells or 20 h after infection with *dl*309 (309) or *dl*331 (331) at a multiplicity of 20 PFU per cell. Interferon-treated cultures first received 1,000 U of human αA interferon per ml 24 h before infection and were refed with the same quantity of fresh interferon after infection. Conditions for the kinase assay and electrophoresis were as described in the legend to Fig. 4. eIF-2α and -β subunits are labeled.

(Fig. 4). This delay is likely to be at least part of the basis for KB cells permissivity. However, it may not be the whole story given the relatively incomplete inhibition of *dl*331 protein synthesis in interferon-treated KB cells compared with 293 cells which contain activated kinase (Fig. 7; see below).

The nature of the kinase-inducing agent generated by *dl*331 infection remains unclear. Since the P1/eIF-2α kinase is known to be induced by interferon, we assayed for interferon production by the *dl*331-infected cultures. The different growth reponse of *dl*331 in 293 and KB cells could be explained easily if 293 cells produced interferon subsequent to infection while KB cells did not. However, no interferon could be detected in the medium of *dl*331-infected KB or 293 cells by a plaque reduction assay, and antibody to β interferon did not inhibit the appearance of kinase activity. It is unlikely that extracellular interferon plays a role. Apparently, a *dl*331 product induces the kinase but not the interferon.

Even though kinase expression exhibited a delay in KB cells compared with 293 cells subsequent to *dl*331 infection (Fig. 4), it was efficiently induced and activated in both cell types when interferon was administered to cultures prior to infection with the VAI mutant (Fig. 7). Curiously, however, *dl*331-specific late polypeptide synthesis (Fig. 8) and yield (Table 1) were much more severely depressed in 293 and KB cells, even though both cells contained activated kinase, as judged by in vitro assays. There is a precedent for this observation. Jacobsen et al. (5) have monitored protein synthesis in extracts of interferon-treated mouse L cells after the addition of a synthetic dsRNA which activated kinase but not (2′-5′)-oligoadenylate synthetase. Activated kinase led to phosphorylation of eIF-2α but did not inhibit protein synthesis. In a similar vein, there have been several reports that viruses normally sensitive to interferon such as reovirus

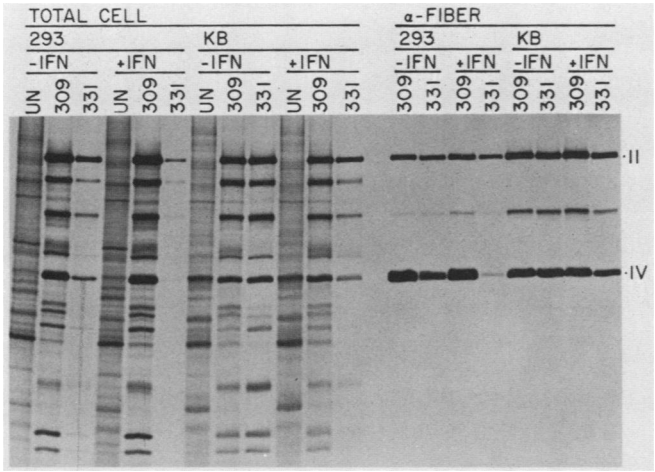

FIG. 8. Electrophoretic analysis of polypeptides synthesized in interferon-treated (+IFN) or untreated (−IFN) cells infected with mutant or wild-type viruses. The 293 or HeLa cells were infected with *dl*309 (309) or *dl*331 (331) at a multiplicity of 20 PFU per cell (UN, uninfected cells). Interferon treatment was as described in the legend to Fig. 7. Cells were labeled for 60 min with [^{35}S]methionine (50 μCi/ml) 22 h after infection. Extracts were prepared and either subjected to electrophoresis directly (total cell) or fiber polypeptide was imunoprecipitated (α-fiber) with a polyclonal antiserum before electrophoresis in a 12.5% polyacrylamide gel containing 0.1% SDS. The two polypeptides which migrated more slowly than the fiber (IV) polypeptide coprecipitated in a nonquantitative fashion through their association with fiber.

TABLE 1. Influence of interferon on virus yield in 293 as compared with KB cells

Virus	PFU/ml in[a]:			
	293 cells		KB cells	
	− IFN	+ IFN	− IFN	+ IFN
*dl*309	2.1×10^8	2.1×10^8	3.0×10^8	3.1×10^8
*dl*331	3.7×10^6	4.2×10^5	3.0×10^8	1.2×10^8

[a] Cells were infected at a multiplicity of 3 PFU/per cell and harvested 48 h later. Virus yield was measured by plaque assay on 293 cells. Interferon (IFN)-treated cultures received 1,000 U of human lenkocyte αA interferon 24 h before infection and were refed with interferon-containing medium subsequent to infection.

(19) and encephalomyocarditis virus (2) can propagate under certain conditions in interferon-treated cells. These cells clearly contained induced kinase, but it was not demonstrated to be activated.

Thus, our results and those of others argue that activation of the interferon-induced kinase and concomitant phosphorylation of eIF-2α is not necessarily sufficient to severely inhibit protein synthesis. Perhaps activated kinase fails to substantially inhibit protein synthesis in cells which fortuitously contain high levels of eIF-2B. Thus, more extensive phosphorylation of eIF-2α than normally observed would be required to trap all available eIF-2B in nonrecyclable complexes with eIF-2-GDP. In this case, then, KB cells would contain higher levels of eIF-2B than 293 cells. Alternatively, another factor may exist that is necessary for translational inhibition via the P1/eIF-2α kinase pathway. If this were true, KB cells must lack this as yet to be identified factor.

ACKNOWLEDGMENTS

We thank S. Pestka (Roche Institute of Molecular Biology) for gifts of human αA interferon and antibody to β interferon, and C. Anderson (Brookhaven National Laboratory) for a supply of polyclonal antibody to the adenovirus type 2 fiber polypeptide.

This work was supported by grant MV-45 from the American Cancer Society. J.K. is a predoctoral trainee supported by Public Health Service grant GM07388 from the National Institute of General Medical Sciences, R.J.S. is a postdoctoral fellow supported by Public Health Service grant CA 07222 from the National Cancer Institute, and T. Shenk is an American Cancer Society Research Professor.

LITERATURE CITED

1. **Crouch, D., and B. Safer.** 1984. The association of eIF-2 with met-tRNA, or EIF-2B alters the specificity of eIF-2 phosphatase. J. Biol. Chem. **259**:10363–10368.
2. **Epstein, D. A., C. W. Czarniecki, H. Jacobsen, R. M. Friedman, and A. Panet.** 1981. A mouse cell line, which is unprotected by interferon against lytic virus infection, lacks ribonuclease F activity. Eur. J. Biochem. **118**:9–15.
3. **Graham, F. L., J. Smiley, W. C. Russell, and R. Nairu.** 1977. Characteristics of a human cell line transformed by DNA from human adenovirus type 5. J. Gen. Virol. **36**:59–72.
4. **Hearing, P., and T. Shenk.** 1983. The adenovirus type 5 E1A transcriptional control region contains a duplicated enhancer element. Cell **33**:695–703.
5. **Jacobsen, H., D. A. Esptein, R. M. Friedman, B. Safer, and P. F. Torrence.** 1983. Double-stranded RNA-dependent phosphorylation of protein P1 and eukaryotic initiation factor 2α does not correlate with protein synthesis inhibition in a cell-free system from interferon-treated mouse L cells. Proc. Natl. Acad. Sci. USA **80**:41–45.
6. **Jagus, R., W. F. Anderson, and B. Safer.** 1981. The regulation of initiation of mammalian protein synthesis. Prog. Nucleic Acid

Res. Mol. Biol. **25**:127–185.
7. **Jones, N., and T. Shenk.** 1979. Isolation of Ad5 host range deletion mutants defective for transformation of rat embryo cells. Cell **17**:683–689.
8. **Kitajewski, J., R. J. Schneider, B. Safer, S. M. Munemitsu, C. E. Samuel, B. Thimmappaya, and T. Shenk.** 1986. Adenovirus VAI RNA antagonizes the antiviral action of interferon by preventing activation of the interferon-induced eIF-2α kinase. Cell **45**:195–200.
9. **Lebleu, B., G. C. Sen, S. Shaila, B. Cabrer, and P. Lengyel.** 1976. Interferon, double-stranded RNA and protein phosphorylation. Proc. Natl. Acad. Sci. USA **73**:3107–3111.
10. **Mathews, M.** 1975. Genes for VA-RNA in Ad2. Cell **6**:223–229.
11. **O'Malley, R. P., T. M. Mariano, J. Siekierka, and M. B. Mathews.** 1986. A mechanism for the control of protein synthesis by adenovirus VA RNA_I. Cell **44**:391–400.
12. **Pettersson, U., and L. Philipson.** 1975. Location of sequences on the adenovirus genome coding for the 5.5S RNA. Cell **6**:1–4.
13. **Reich, P. R., J. Rose, B. Forget, and S. M. Weissman.** 1966. RNA of low molecular weight in KB cells infected with Ad2. J. Mol. Biol. **17**:428–439.
14. **Reichel, P. A., W. C. Merrick, J. Siekierka, and M. B. Mathews.** 1985. Adenovirus VA RNA_I regulates the activity of a protein synthesis initiator factor. Nature (London) **313**:196–200.
15. **Roberts, W. K., A. Hovanessian, R. E. Brown, M. J. Clemens, and I. M. Kerr.** 1976. Interferon-mediated protein kinase and low-molecular-weight inhibitor of protein synthesis. Nature (London) **264**:477–480.
16. **Safer, B.** 1983. 2B or not 2B: regulation of the catalytic utilization of eIF-2. Cell **33**:7–8.
17. **Samuel, C. E.** 1981. Procedures for measurement of phosphorylation of ribosome-associated proteins in interferon-treated cells. Methods Enzymol. **79**:168–178.
18. **Samuel, C. E., D. A. Farris, and D. A. Eppstein.** 1977. Mechanisms of interferon action. Kinetics of interferon action in mouse L_{929} cells: translation inhibition, protein phosphorylation, and mRNA phosphorylation and degradation. Virology **83**:56–71.
19. **Samuel, C. E., and G. S. Knutson.** 1981. Mechanism of interferon action: cloned human leukocyte interferons induce protein kinase and inhibit vesicular stomatitis virus but not reovirus replication in human amnion cells. Virology **114**:302–306.
20. **Sarnow, P., Y. S. Ho, J. Williams, and A. J. Levine.** 1982. Adenovirus E1b-58kd tumor antigen and SV40 large tumor antigen are physically associated with the same 54kd cellular protein in transformed cells. Cell **28**:387–394.
21. **Schneider, R. J., B. Safer, S. M. Munemitsu, C. E. Samuel, and T. Shenk.** 1985. Adenovirus VAI RNA prevents phosphorylation of the eukaryotic initiation factor 2 alpha subunit subsequent to infection. Proc. Natl. Acad. Sci. USA **82**:4321–4325.
22. **Schneider, R. J., C. Weinberger, and T. Shenk.** 1984. The adenovirus VAI RNA functions during initiation of translation in virus-infected cells. Cell **37**:291–298.
23. **Siekierka, J., T. M. Mariano, P. A. Reichel, and M. B. Mathews.** 1985. 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. Proc. Natl. Acad. Sci. USA **82**:1959–1963.
24. **Soderlund, H., U. Pettersson, B. Vennstrom, L. Philipson, M. B. Mathews.** 1976. A new species of virus-coded low molecular weight RNA from cells infected with Ad 2. Cell **7**:585–593.
25. **Tahara, S. M., J. A. Traugh, S. B. Sharp, B. Safer, and W. C. Merrick.** 1978. Effect of hemin on site-specific phosphorylation of eukaryotic initiation factor 2. Proc. Natl. Acad. Sci. USA **75**:789–793.
26. **Thimmappaya, B., C. Weinberger, R. J. Schneider, and T. Shenk.** 1982. Adenovirus VAI RNA is required for efficient translation of viral mRNAs at late times after infection. Cell **31**:543–551.
27. **Traugh, J. A., S. M. Tahara, S. B. Sharp, B. Safer, and W. C. Merrick.** 1976. Factors involved in initiation of haemoglobin synthesis can be phosphorylated *in vitro*. Nature (London) **263**:163–165.
28. **Weinmann, R., H. J. Raskas, and R. G. Roeder.** 1974. Role of DNA-dependent RNA polymerases II and III in transcription of the adenovirus genome late in productive infection. Proc. Natl. Acad. Sci. USA **71**:3426–3430.
29. **Zilberstein, A., P. Federmann, L. Schulman, and M. Revel.** 1976. Specific phosphorylation of a protein associated with ribosomes of interferon-treated mouse L cells. FEBS Lett. **68**:119–124.