Deletion of the vaccinia virus K3L gene, a homologue of the α subunit of protein synthesis initiation factor 2, has been reported to reduce the ability of the virus to grow in interferon-treated cells (Beattie, E., Tattaglia, J., and Paolletti, E. (1991) Virology 183, 419–422). Purified recombinant K3L gene product, pK3, has potent effects on activation of double-stranded (ds) RNA-dependent, initiation factor-2α (eIF-2α)-specific protein kinase (PKR) in vitro reactions. Recombinant pK3 prevents the inhibition of protein synthesis by dsRNA in a cell-free translation system from rabbit reticulocytes at levels equal to, or lower than, the level of endogenous eIF-2α. In the cell-free translation system, pK3 exerts its effects at all dsRNA concentrations tested, by preventing phosphorylation of eIF-2α. In addition, pK3 reduces the autophosphorylation of immunopurified PKR, as well as its ability to phosphorylate the α subunit of purified eIF-2. At 400 mM NaCl, in vitro translated [35S]methionine-radiolabeled pK3 can be co-immunoprecipitated with human PKR, using a monoclonal antibody to PKR. This tight binding is consistent with a role for PK3 as a pseudosubstrate for the kinase, and identifies the amino-terminal 30% of eIF-2α as the domain recognized by the eIF-2α-specific protein kinases. In addition, the tight binding opens up the possibility of using binding assays to identify functional domains within the kinase and pK3.

Recombinant pK3 also prevents activation of the heme-sensitive eIF-2α-specific protein kinase, eIF-2α-PKh, in both cell-free translation systems as well as in partially purified preparations. This suggests some similarity between the eIF-2α binding domains of the two eIF-2α specific protein kinases.

In animal cells, virus replication may be limited by the action of interferon (reviewed in Refs. 2 and 3). One interferon-inducible enzyme involved in host defense is a double-stranded (ds) RNA-dependent, eIF-2α-specific protein kinase, recently termed PKR2 (4, 5). PKR is a serine/threonine protein kinase in the same kinase subfamily as eIF-2α-PKh and GCN2 (6–8). This enzyme has been variously referred to in the literature as eIF-2α-PKh (9), p68 kinase (10, 11), DAI (12), and dsRNA-PK (13). Levels of PKR increase 3–10-fold after exposure of cells to interferon, and the enzyme exists in an inactive form prior to virus infection (13). Activation of PKR has been found to occur upon infection by many viruses (14–21) and results in phosphorylation of eIF-2α (16, 19–21). This in turn leads to an inhibition of protein synthesis by disrupting the GTP/GDP exchange cycle required for eIF-2 function (22). However, several animal viruses, including adenovirus (reviewed in Ref. 23), human immunodeficiency virus (24), influenza virus (25–27), polio virus (28), and vaccinia virus (9, 14, 15) have evolved mechanisms to overcome the interferon response by down-regulating the activity of PKR, a characteristic that reduces virus susceptibility to interferon (reviewed in Refs. 29–31). Some viruses down-regulate PKR by the generation of inhibitors of kinase function. For instance, in adenovirus-infected cells, there is production of a small RNA, VA1 RNA, that binds to PKR and prevents its activation (23). Influenza virus infection activates a cellular protein that inhibits PKR activation by preventing kinase autophosphorylation (26–27). Reovirus-infected cells produce a gene product, p3, that inhibits PKR in vitro by binding to dsRNA (32).

Vaccinia virus also produces a protein inhibitor of PKR function (9, 14, 15). Extracts of vaccinia virus-infected cells contain a protein inhibitor of kinase function that appears to act by interacting with dsRNA (9, 14, 15). This activity may correspond to a 25-kDa dsRNA-binding protein, the gene product of E3L (33, 34). In addition, an accumulation of evidence implicates the vaccinia virus gene, K3L, as playing a significant role in the down-regulation of PKR activation. Gene K3L, initially named K2L in the WR strain (35), encodes an 88-amino acid polypeptide that has a 28% identity and 72% similarity to the amino terminus of eIF-2α (1, 36). The region of overlap with the larger eIF-2α covers the PKR

1The abbreviations used are: ds, double-stranded; DTT, dithiothreitol; eIF, initiation factor; eIF-2α-PKh, heme-sensitive eIF-2α kinase; PKR, dsRNA-dependent eIF-2α kinase; pK3, vaccinia virus K3L gene product; pK3, recombinant pK3; PAGE, polyacrylamide gel electrophoresis; VSEIF, vertical slab isoelectric focusing; SKIF, specific kinase inhibitor factor; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonic acid; Triene, N-tris(hydroxymethyl)methylglycine.

2The nomenclature used for PKR is that recommended by informal agreement by workers in the field (4, 5). Nomenclature used for initiation factors is that recommended by the Nomenclature Committee of the International Union of Biochemists (52).
phosphorylation site on eIF-2α at residue 51 (I), although pK3 does not have a phosphorylatable serine at this position (37). A K3L deletion mutant of vaccinia virus has been reported to have increased sensitivity to interferon (I), and co-transfection of the K3L gene into COS cells enhances translation of a reporter gene by preventing eIF-2α phosphorylation and activation of PKR (37).

The experiments described in this paper look at the in vitro activity of purified recombinant pK3 in a cell-free translation system from rabbit reticulocytes, as well as its effects on immunopurified PKR. The reticulocyte cell-free translation system contains two eIF-2α-specific protein kinases, PKR, as well as a heme-sensitive eIF-2α-specific protein kinase (eIF-2α-PK3). We report that pK3 prevents eIF-2α phosphorylation and thus prevents and reverses the inhibition of translation resulting from the activation of PKR. In addition, we have found that pK3 prevents the autophosphorylation of immunopurified PKR, as well as its ability to phosphorylate purified eIF-2α. Similarly, we have found that pK3 prevents the inhibition of translation by PKR, although higher concentrations of pK3 are necessary than the inhibition of translation by activation of eIF-2α-PKh, mRNA upstream of the initiation codon of K3L, allowing efficient erase chain reaction amplification using the primers 5’-GGGGCCATGGTTGCATTTTGTTATTCG-3’ and 5”GGGGGG- between the NcoI and BamHI restriction sites. The sequence of the gene was confirmed by dideoxy DNA sequencing (40). The vector, pTM1 also contains the T7 polymerase promoter and as monolayers in Dulbecco’s modified Eagle’s medium supplemented amino acids minus valine, [14C]valine (50 pM, 120 mCi/mmol, Du Pont-New England Nuclear), 10 μM phosphocreatine, 2 units/ml creatine phosphokinase, and 20 μM hemin. The translation mix contained approximately 20 pmol of ribosomes/ml, 55-65 pmol of eIF-2α, and exhibited an eIF-2α synthetase activity equivalent to 1 pmol of globin/pm of ribosomes/min at 30 °C.

Evaluation of eIF-2α Phosphorylation State in Reticulocyte Translation System—Reticulocyte translation system was incubated under appropriate experimental conditions, in the absence of radioactive amino acids. After incubation, 5-μl aliquots were added to 2.5 ml of buffer/ml of original cell culture. This material was centrifuged for 15 min at 10,000 rpm in a Beckman JA-20 rotor, at 0 °C. The pellet was recovered and solubilized in 1 ml KCl, 50 mM HEPES, pH 7.5, 1 mM EDTA, 0.1 mM DTT, 50 μg/ml of T7 polymerase, and 1 μM DTT, 10% glycerol. This material was extensively dialyzed against the same buffer at 4 °C overnight. The purified protein was clarified with BPA-1000 (5 μl/ml) (Toso-Haas), followed by microcentrifugation. This protocol gave yields of pK3, of approximately 1 mg/100 ml of cell culture and was essentially homogeneous. Protein concentration of purified pK3, was determined by the method of Bradford (42), using a kit from Bio-Rad. Molarity was calculated from protein concentration using a molecular weight of 10,556 derived from the deduced amino acid sequence (1). The purified pK3, was shown to phosphorylate ATPase (activities not shown). Note that the use of M, 10,000-12,000 cut-off dialysis tubing results in significant loss of pK3.

Rabbit Reticulocyte Translation System—Rabbit reticulocyte lysate was prepared and used as described (43). In vitro translation reactions were performed with the addition of 100 mM KCl, 0.5 mM MgCl2, 150 μM amino acids minus valine, [14C]valine (50 μM, 120 mCi/mmol, Du Pont-New England Nuclear), 10 μM phosphocreatine, 2 units/ml creatine phosphokinase, and 20 μM hemin. The translation mix contained approximately 20 pmol of ribosomes/ml, 55-65 pmol of eIF-2α, and exhibited an eIF-2α synthetase activity equivalent to 1 pmol of globin/pm of ribosomes/min at 30 °C.

* R. Jagus and K. Carroll, unpublished observations.
mm EDTA, 10% glycerol, 10 µg/ml aprotinin, 5 µg/ml leupeptin, essentially as described by Katze et al. (46).
eIF-2α-specific Protein Kinase Assays—Immunopurified PKR, re-suspended in 100 mM KCl, 25 mM HEPES, pH 7.2, 10% glycerol, 0.2 mg/ml bovine serum albumin, 10 µg/ml aprotinin, 5 µg/ml leupeptin, 2 mM MgCl2, 1 mM MnCl2, and 5 mM [γ-32P]ATP (50-100 Ci/mmol, Du Pont-New England Nuclear), and where indicated, 4 pmol of purified rabbit eIF-2, essentially as described (28). The reaction was incubated at 30 °C for 15 min and stopped by the addition of one volume of a two-fold concentration of SDS-PAGE sample buffer, boiled for 5 min, and analyzed by SDS-PAGE.

Polyacrylamide Gel Electrophoresis (SDS-PAGE)—Samples were analyzed by 10% SDS-PAGE, essentially as described (47), with the exception of those containing pK3, which were analyzed by SDS-PAGE using a Tris/Tricine buffer system for the resolution of smaller polypeptides as described (48).

Transcription/Translation of [35S]Methionine-radio labeled pK3,—[35S]Methionine-radio labeled pK3 was produced in an mRNA-dependent reticulocyte translation system, from pTM1/K3L DNA, using the Promega TNT-coupled transcription/translation system, containing 50 µCi/100 µl [35S]methionine. pTM1 puts the 5′ untranslated region of encephalomyocarditis virus upstream of the initiation codon, allowing efficient translation without capping of mRNA (39).

RESULTS
Effects of pK3, on the Activity of the Cell-free Rabbit Reticuloocyte Translation System—Fig. 1 (panel A) shows the ability of increasing concentrations of pK3, to prevent translational inhibition in a reticulocyte translation system caused by activation of the endogenous dsRNA-dependent eIF-2α-specific protein kinase. The addition of poly(I)-poly(C) (Pharmacia LKB Biotechnology Inc.) at 125 ng/ml reduces the rate of protein synthesis to approximately 5% of control, after a lag of 15–20 min. This reduces the incorporation of [14C]valine into trichloroacetic acid-precipitable material to approximately 50% of control value after a 30-min incubation. The inhibition reflects the inactivation of eIF-2 resulting from the activation of PKR. The addition of pK3, prevents the inhibition, at levels between 2–4 pmol/100 µl. This is similar to the levels of endogenous eIF-2, estimated to be between 5.5 and 6.5 pmol/100 µl in reticulocyte lysate by immunoblotting (49). To ensure that the effect on translation was due to the added pK3, and not due to a trace contaminant from the bacterial cells, an equivalent protein fraction was generated from untransformed cells, as well as from cells containing vector only. Such protein fractions had no effect on the rabbit reticulocyte translation system (data not shown). In addition, pK3, that was boiled for 10 min lost its ability to prevent dsRNA translation inhibition (data not shown).

Fig. 1 (panel B) demonstrates that in addition to preventing PKR activation in the reticulocyte translation system, pK3, can also rescue translation in the reticulocyte translation system after inhibition by dsRNA has been established. In this experiment, dsRNA (poly(I)-poly(C)) was added to a final concentration of 125 ng/ml at time zero. In the presence of dsRNA, protein synthetic rates proceed at control rates for 15–20 min and then decline to reach a final rate of about 5% of control values. At 19 min, pK3, was added, to 5 pmol/100 µl, and reversed the inhibition of protein synthesis to give rates approaching those of control, after a lag of approximately 6 min. These data suggest that pK3, may act as a pseudosubstrate for PKR, and that the turnover rate of the activated state of PKR in the rabbit reticulocyte system is approximately 6 min.

The Effect of pK3, on Translation Is Not Dependent on dsRNA Concentration—The absolute concentrations of dsRNA required to activate PKR vary with the type of dsRNA, its molecular weight, and the system under study. Fig. 2 (panels A and B) shows the effects of added pK3, (5 pmol/100 µl) over a wide range of dsRNA (poly(I)-poly(C)) on the translational activity of the rabbit reticulocyte translation system. The batch of poly(I)-poly(C) used in these studies began to inhibit at 12.5 ng/ml, and gave maximum inhibition at 50 ng/ml; not until 5 µg/ml of dsRNA did this level of inhibition begin to decrease. The effect of two levels of pK3, 4 and 18 pmol/100 µl, was examined over this extended concentration range of dsRNA. Both levels of pK3, prevented the inhibition of dsRNA on translation over this entire range. The function of pK3, is independent of dsRNA concentration, unlike the activity previously described as specific kinase inhibitory factor, or SKIF (9, 15). SKIF has been shown to displace the concentration dependence of PKR for dsRNA, and probably corresponds to the vaccinia virus E3L gene product, a dsRNA-binding protein (33, 34). The

Fig. 1. Effect of pK3, on translation in a cell-free translation system from rabbit reticulocytes. Panel A, cell-free extracts of rabbit reticulocytes were incubated under standard conditions, as described under "Materials and Methods," in the absence (open circles) or presence (filled circles) of dsRNA (poly(I)-poly(C), 125 ng/ml). pK3, was added as indicated. Protein synthetic activity was measured by [14C]valine incorporation into trichloroacetic acid-precipitable material after a 30-min incubation at 30 °C. Panel B, cell-free extracts of rabbit reticulocytes were incubated under standard conditions, as described under "Materials and Methods," and supplemented as follows: open squares, no additions, filled squares, 18 pmol of pK3/100-µl reaction; open triangles, 125 ng/ml dsRNA; filled triangles, 125 ng/ml dsRNA (poly(I)-poly(C)) and 18 pmol of pK3/100-µl reaction added at 19 min (indicated by vertical arrow). Incubation was at 30 °C, and protein synthetic activity was measured by [14C]valine incorporation into trichloroacetic acid-precipitable material at the times indicated.
Poly(C)) was added as indicated. Protein synthetic activity was measurable material after a 30-min incubation at 30 °C.

In a cell-free rabbit reticulocyte translation system, extracts of rabbit reticulocytes were incubated under standard conditions, as described under “Materials and Methods,” in the presence or absence of poly(I)-poly(C) was added as indicated. Protein synthetic activity was measured by \([^{14}C]\)valine incorporation into trichloroacetic acid-precipitable material after a 30-min incubation at 30 °C.

The above data demonstrate that pK3 does not function as a dsRNA-binding protein and is not equivalent to the activity previously described as SKIF.

Effect of pK3, on eIF-2a-PKh in the Rabbit Reticulocyte System—The reticulocyte translation system contains another eIF-2a-specific kinase, eIF-2a-PKh, a heme-sensitive kinase, which is activated by incubation in the absence of added hemin. It was of interest to know whether pK3, was specific for PKR or whether it would also have an effect on the activity of eIF-2a-PKh. Fig. 3 shows the effect of pK3, on translational inhibition by hemin deprivation in the reticulocyte translation system. In the absence of hemin, protein synthesis continued at control rates for approximately 3-5 min, after which the rate decreased rapidly to give a final rate of approximately 5% of control. After a 30-min incubation, this resulted in the reduction of \([^{14}C]\)valine incorporation to approximately 30% of control. This reflects the inactivation of eIF-2 resulting from the activation of eIF-2a-PKh. Added pK3, also prevented translational inhibition by hemin deprivation, but much higher levels were needed than for preventing inhibition caused by dsRNA addition. Approximately 90-100 pmol/100 μl of pK3, were needed to prevent the inhibition of translation caused by hemin depletion, a concentration approximately 20-fold higher than that needed to prevent translational inhibition by dsRNA. These data suggest that although pK3, is acting as a competitive inhibitor for both kinases, it is a better competitor for PKR than it is for eIF-2a-PKh.

The ability of pK3 to prevent activation of eIF-2a-PKh as well as PKR is also distinct from the characteristics of SKIF, which is unable to prevent activation of eIF-2a-PKh during the incubation of a reticulocyte translation system in the absence of hemin (9).

pK3, Prevents eIF-2a Phosphorylation in the Reticulocyte Translation System—The effects of pK3, on activation state of the eIF-2a-specific protein kinases were not measured directly, but its effects on eIF-2a phosphorylation state in the reticulocyte translation system are shown in Fig. 4. The phosphorylation state of eIF-2a was determined by quantitative immunoblotting after vertical slab isoelectric focusing (VSIEF), which separates phosphorylated and nonphosphorylated forms of eIF-2a (44). The ratio of intensities of these two bands gives a direct measure of the steady state phosphorylation state of eIF-2a, as previously demonstrated (44).

In a reticulocyte translation system incubated with hemin and without dsRNA (lane 1), eIF-2a was not significantly phosphorylated. After incubation with 125 ng/ml poly(I)-poly(C) for 30 min (lane 2), eIF-2a became phosphorylated to a steady state level of approximately 25%. This could be prevented (lane 3) by the addition of pK3, (5 pmol/100 μl). Similarly, incubation in the absence of hemin led to phosphorylation of eIF-2a (lane 4), and although pK3, at 5 pmol/100 μl was insufficient to prevent this (lane 5), at 50 pmol/100 μl pK3, was able to significantly reduce this phosphorylation (lane 6).

pK3, Prevents Activation and Inhibits Activity of Immunopurified PKR—PKR can be immunopurified from cell extracts using monoclonal antibodies covalently bound to CL-4B Sepharose (28, 46). Fig. 5 (panel A) shows the effect of pK3, on PKR immunopurified from 293 cells. The kinase activity of PKR so purified from interferon-treated 293 cells was assessed by the level of autophosphorylation, as well as by its ability to phosphorylate its substrate, eIF-2a, by measuring the incorporation of \([^{32}P]\)phosphate from \([γ-^{32}P]\)ATP into PKR. Fig. 5 (panel A, lanes 1 and 2) shows the autophosphorylation of 293 cell PKR in the presence and absence of 0.2 μg/ml poly(I).poly(C). The autophosphorylated kinase appeared as a band of 68 kDa on the autoradiographs. There was little stimulation in the level of autophosphorylation of PKR incubated with dsRNA. This has been observed by other

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\text{pK3, Prevents phosphorylation of eIF-2a in rabbit reticulocyte translation system. Cell extracts from rabbit reticulocytes were incubated for 30 min under standard conditions, as described under "Materials and Methods." In the presence or absence of 125 ng/ml dsRNA (poly(I).poly(C)), 20 μM hemin, or 5 (+) or 50 (+) pmol of pK3, /100 μl, as indicated in the figure. Samples were fractionated by VSIEF, transferred to Immobilon-P, and subjected to immunoblotting, as described (44).}
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\text{pK3, prevents phosphorylation of eIF-2a in rabbit reticulocyte translation system.}
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investigators and may suggest that PKR from 293 cells is already activated. The addition of PK3, caused only a slight reduction in PKR autophosphorylation (lanes 4, 5, and 7). When eIF-2 was present in the reaction (lane 3), the α subunit became phosphorylated. With the addition of PK3, the phosphorylation of eIF-2α was blocked (lanes 6 and 8).

* M. Katze, personal communication.

In Fig. 5 (panel B) the effect of poly(I)-poly(C) and PK3, on the function of PKR from interferon-treated HeLa cells was examined by the autophosphorylation of PKR. In contrast to PKR from 293 cells, PKR from HeLa cells does respond to dsRNA by increased autophosphorylation. As shown in lane 1, PKR from HeLa cells was not maximally autophosphorylated, and autophosphorylation could be stimulated in vitro by incubation with poly(I)-poly(C) from 0.05–0.2 μg/ml, as seen in lanes 2–4. The dsRNA-stimulated autophosphorylation was significantly reduced by the inclusion of 5 or 20 pmol pK3, as shown in lanes 5 and 6, respectively. Lane 7 shows that extracts from bacteria transformed with vector not containing the K3L insert had no effect on kinase autophosphorylation. Similarly, lane 8 shows that PKR autophosphorylation could not be reduced by pK3, inactivated by heat denaturation.

**pK3.** *Also Inhibits the Activity of Partially Purified eIF-2α-PKR.*—Fig. 5 (panel C) shows the effect of pK3, on the activity of partially purified eIF-2α-PKR, which has a molecular weight of 90,000. Lane 2 shows that in the absence of pK3, added eIF-2α was phosphorylated. Notice in lane 1 that the eIF-2α-PKR preparation contained some contaminating eIF-2α. Lane 4 shows that the addition of 50 pmol of pK3, significantly reduced the phosphorylation of eIF-2α, although kinase autophosphorylation was not appreciably affected.

**pK3.** *Can Be Co-immunoprecipitated with PKR—*Because the sequence of the K3L gene shows homology with that of the eIF-2α gene, and because pK3 has such potent inhibitory effects on eIF-2α kinase function, it was anticipated that pK3 might function by direct interaction with PKR and may exhibit tight binding. To determine this, [35S]methionine-radiolabeled pK3, was incubated with eIF-2α-PK-R bound to monomodal antibodies covalently bound to CL-4B Sepharose. For this experiment, [35S]methionine-labeled pK3, was produced by translation of an in vitro produced transcript in an mRNA-dependent reticulocyte translation system, using the K3L gene in pTM1. After [35S]methionine-radiolabeled pK3, production, the translation mix was incubated at 30 °C for 5 min with PKR pre-bound to antibody bound to CL-4B Sepharose. The resin was washed extensively with buffer containing either 400 mM or 1 M NaCl. Fig. 5D (lane 1) shows that at 1 M NaCl, [35S]methionine-radiolabeled pK3, did not bind well to the resin. However, lane 2 shows that at 400 mM salt [35S]methionine-radiolabeled pK3, binds sufficiently tightly to be co-immunoprecipitated. Lanes 3 and 4 demonstrate that in the absence of PKR, there is no appreciable binding of [35S]methionine-radiolabeled pK3, to the resin. The endogenous rabbit PKR does not contribute to [35S]methionine-radiolabeled pK3, binding to the resin, since it cannot interact with the monoclonal antibodies to human PKR.

**DISCUSSION**

The vaccinia virus K3L gene product, pK3, is a small polypeptide that is homologous to the α subunit of eIF-2. In this investigation, we have shown that recombinant pK3 prevents the activation of PKR in vitro, either in cell-free translation systems, or using immunopurified PKR, thereby preventing eIF-2α phosphorylation. pK3 exerts its effects by tightly binding to the kinase. pK3 is presumed to bind within the eIF-2α binding domain of PKR and these studies suggest that the amino-terminal 30% of eIF-2α contains structures critical for interaction with the kinase. Since pK3, also exerts its effects on eIF-2α-PKR, it seems likely that the two kinases have eIF-2α binding domains that share some sequence homology, but are sufficiently distinct to give different affinities for pK3. The effects of pK3, on autophosphorylation of PKR...
suggest that the eIF-2α binding site and the autophosphorylation site are closely associated in the three-dimensional structure of this kinase.

The characteristics of pK3 demonstrated in this work are clearly distinguishable from the activity previously described as SKIF (9), currently thought to be the vaccinia virus E3L gene product. Unlike SKIF, pK3 is unable to shift the concentration dependence of PKR for dsRNA. Also in contrast to SKIF, pK3 is able to inhibit the activity of eIF-2α-PKh. In the preparation of SKIF previously described (9), it is probable that most of the pK3 present in the cell extract was lost during dialysis prior to use, due to its small size.

Recent findings identify PKR not only as a component of the host defense system, but also as a potent anti-oncogene (50, 51). Little is currently known about this anti-oncogenic activity. Furthermore, little is known about how PKR interacts with its substrate(s). The ability of pK3 to bind tightly to the kinase presents the possibility of developing a binding assay to examine the functional domains of eIF-2α-specific protein kinases as well as pK3. By extrapolation, this should also offer some insight into the structure-function relationships of eIF-2α. Such studies should increase our understanding of the role of PKR in preventing or reversing cellular transformation.

The studies presented in this paper show that pK3 has potent effects on PKR in vitro. Earlier studies have shown that the K3L gene transiently expressed in COS cells also has potent effects on PKR. However, the significance of the gene on the ability of vaccinia virus to grow in interferon-treated cells remains unclear. The K3L gene is expressed at early times in vaccinia virus infection (35). However, the E3L gene, another vaccinia virus early gene, also has potent effects on PKR activity, both in vitro and in transfecte COS cells (34). The possibility arises that vaccinia virus has evolved two mechanisms to counteract the interferon-induced antiviral host cell response to infection.

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