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SHORT COMMUNICATION

Human PKR Transfected into Murine Cells Stimulates Expression of Genes under Control of the HIV1 or HTLV-I LTR

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We have analyzed the effect of transfection into murine NIH/3T3 cells of the human dsRNA-activated kinase PKR on the expression of the β-galactosidase reporter gene, placed under control of the HIV1 or the HTLV-I LTR. β-Galactosidase expression is stimulated when the reporter plasmids are cotransfected with wild-type PKR but inhibited when cotransfected with a catalytically inactive mutant PKR. In the case of HIV1, β-galactosidase expression was not stimulated when cotransfection was carried out with PKR harboring mutations in the dsRNA binding domains, indicating that stimulation depends on the classical mode of PKR activation through dsRNA binding. In contrast, the dsRNA binding mutants of PKR could still partially stimulate β-galactosidase expression from the HTLV-I LTR, suggesting that PKR activation in this case may involve different/additional mechanisms. These results show that, in addition to the known down-regulation of protein synthesis through eIF2 phosphorylation, PKR can also positively stimulate gene expression *in vivo*, most probably through phosphorylation of a substrate distinct from eIF2.

The double-stranded RNA-activated protein kinase PKR is a serine/threonine kinase that is present at basal levels in most cells and can be induced after interferon treatment. The function of PKR is regulated by the presence in the cells of dsRNA molecules, ssRNA presenting internal dsRNA regions or other polyanionic molecules (*1*). Once activated by dsRNA, which triggers its autophosphorylation, PKR phosphorylates the α subunit of the initiation factor eIF2, leading to inhibition of protein synthesis (*2, 3*).

PKR plays an important role in the antiviral and antiproliferative actions of interferon. Cell lines expressing the wt PKR caused partial inhibition of encephalomyocarditis virus replication. This inhibition, which was not observed in cell lines expressing a catalytically inactive mutant of PKR, coincided with *in vivo* phosphorylation of both PKR and its substrate, eIF2. Thus, PKR can be directly implicated in one of the mechanisms leading to the antiviral action of interferon (*4*). In addition, a cell line expressing a mutant of PKR, lacking the eIF2 binding region (*5*), and cell lines constitutively expressing catalytically inactive mutant PKR developed tumors in nude mice. Because tumors were not formed by injection of cells expressing wt PKR, these results collectively demonstrated that mutant PKR can function as a dominant oncogene (*6*). It is not clear whether this latter function involves eIF2 phosphorylation or if another substrate is required. In this regard, it has been recently demonstrated that PKR can also phosphorylate IκB, the NF-κB inhibitor, thus liberating NF-κB activity and implicating PKR in the signal transduction pathway of specific genes (*7, 8*).

Since PKR binds to, and can be activated by, double-stranded RNA molecules, it represents an important threat for many viruses that synthesize and are dependent on double-stranded RNA for replication and transcription. Systematic analysis of the relationship between viruses and PKR has revealed that many viruses can specifically escape or counteract PKR action. For instance, inhibition of PKR can be obtained (a) by direct binding to high concentrations of viral dsRNA [adenovirus VA1 (*9*), Epstein–Barr EBERs (*10*)], (b) by competition with viral proteins for the dsRNA activator [vaccinia E3L protein (*11*), reovirus σ3 protein (*12*)], (c) by interference in the interaction with the substrate eIF2 [vaccinia K3L protein (*13*)], (d) by virus-induced synthesis of cellular protein inhibitors for PKR [poliovirus-induced cellular protease (*14*), influenza virus-induced TPRp58 (*15*)], or (e) by PKR compartmentalization [EMCV (*16*)].

The interactions between PKR and HIV1 have also been analyzed in some detail. All HIV mRNAs contain a double-stranded RNA stem–loop structure, in their 5′

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untranslated leader sequence, which is transcribed with the TAR region of the LTR and located downstream of the site of transcription initiation. Below the loop, there is a three-nucleotide bulge which is necessary for the binding of the HIV1 transactivating Tat protein. The size of the double-stranded stem structure of TAR is sufficiently long to cause activation of PKR but different experimental studies have yielded controversial results. Although it has been clearly demonstrated that TAR binds to PKR *in vitro* (*17*), there are conflicting reports on whether TAR mediates transinhibition of translation through activation of PKR and subsequent eIF2 phosphorylation (*18–21*) or behaves as an inhibitor of PKR by sequestration, like VA1 or EBER RNAs (*22, 23*). The experiments indicating TAR-activation of PKR were all performed *in vitro* while the experiments concluding that TAR inhibited PKR also involved *in vivo* transfection assays. However, all of the latter transfection experiments were based on expression of a reporter gene coupled to, or transfected in the presence of, TAR RNA and did not deal directly with PKR (*22, 23*).

In this study, we investigated the effect of PKR on the expression of a reporter gene (nuclear-targeted β-galactosidase), placed directly under the control of the LTR region of HIV1 (*24*), by transient transfections with different coding sequences for the human PKR. We have compared the properties of the native enzyme (PKR^{wt}) with a catalytically inactive mutant ($PKR^{K\text{-}R296}$) and single substitution mutants in the first dsRNA binding domain of the kinase ($PKR^{G\text{-}A57}$, $PKR^{K\text{-}A60}$, and $PKR^{K\text{-}A64}$). The results show that PKR is able to stimulate gene expression from the HIV1 LTR. Similarly, we also show that PKR is able to stimulate gene expression from the HTLV-I LTR.

In order to observe data resulting exclusively from the simultaneous presence of the LTRs and the different PKR constructs (Fig. 1), we have chosen to perform our assays in murine NIH/3T3 cells since these cells contain extremely low levels of endogenous PKR (*25*). Although expression of the HIV1 LTR is much lower in murine cells than in human cells, preliminary experiments indicated that expression from the pJK2 plasmid could be reproducibly detected in NIH/3T3 cells. When the PKR plasmids were coprecipitated with either 0.5 or 1.5 μg of pJK2, we observed that β-galactosidase expression was enhanced by PKR^{wt} but inhibited in the presence of the catalytically inactive mutant $PKR^{K\text{-}R296}$ (Fig. 2A). Increasing amounts of the PKR^{wt} plasmid (0.5 to 3 μg) cotransfected with 0.5 μg of pJK2 gradually increased the stimulation of expression of pJK2 with a maximum of threefold stimulation obtained for 3 μg of DNA. On the other hand, increasing the concentration of $PKR^{K\text{-}R296}$ gradually inhibited the basal expression of pJK2 to a maximum of twofold inhibition (Fig. 2B).

To confirm this observation, we directly examined the expression of pJK2 in NIH/3T3 clones constitutively expressing the human PKR^{wt} or the $PKR^{K\text{-}R296}$ (*4*). The levels of expression of PKR^{wt} in clone 68.11 and $PKR^{K\text{-}R296}$ in clone 12.3 compared with the control NIH/3T3 clone (Neo) are shown in Fig. 2C. Transfection of pJK2 into these clones resulted in higher expression of β-galactosidase in the clone 68.11 expressing PKR^{wt} than in the control clone Neo and much lower expression in the clone 12.3 expressing the catalytically inactive $PKR^{K\text{-}R296}$ (Fig. 2D). These findings therefore are in accord with the results obtained from cotransfections and indicate clearly that PKR is functionally involved in this process.

PKR consists of two domains, a catalytic domain, located in the carboxy-terminal part of the protein, which is responsible for the kinase activity (*26*), and a regulatory domain, located in the amino-terminal part of the protein. The regulatory domain comprises two basic dsRNA binding motifs (*27, 28*) and a third basic domain which does not bind dsRNA but is required for PKR activity (*29, 30*). Mutation analysis by deletion or by amino acid substitution has defined the amino acids that are critical for dsRNA binding. Specifically, three amino acids (G^{57}, K^{60}, and K^{64}) of the first dsRNA binding domain ($G^{57}RSK^{60}KEAK^{64}NAAAKLAVEIL$) are essential for dsRNA-binding of PKR *in vitro* and expression of a growth suppressor phenotype in yeast (*31, 32*). To determine whether the stimulation of β-galactosidase expression from the HIV1 LTR was specifically mediated by PKR, through its activation by dsRNA, we analyzed the expression of the HIV1 LTR–β-galactosidase in the presence of three PKR^{dsRNA-} mutants: $PKR^{G\text{-}A57}$, $PKR^{K\text{-}A60}$, and $PKR^{K\text{-}A64}$. The results (Fig. 3) show that the expression of β-galactosidase is reduced in the presence of the three PKR^{dsRNA-} mutants. These data clearly establish that the stimulation of β-galactosidase observed when pJK2 is coexpressed with PKR^{wt} is related to activation of PKR from binding to dsRNA molecules.

We have also examined whether the PKR-induced stimulation of gene expression observed for the HIV1 LTR could apply to other promoters. Similar cotransfection experiments were carried out using constructs in which the HIV1 LTR was replaced with the HTLV-I LTR or with the CMV immediate-early promoter upstream of the β-galactosidase gene. The results (Fig. 4A) show that PKR^{wt} stimulates, and catalytically inactive $PKR^{K\text{-}R296}$ inhibits, the expression of β-galactosidase under the control of HTLV-I LTR, similar to the results obtained for the HIV1 LTR. However, when we examined the PKR^{dsRNA-} mutants, we found, in contrast to the HIV1 LTR, that all three mutants stimulated expression of β-galactosidase from the HTLV-I LTR, although to a lesser extent than PKR^{wt}. When the reporter gene was placed under the control of the CMV promoter, there was less striking regulation of expression by PKR. Moreover, β-galactosidase expression was not inhibited in the presence of $PKR^{K\text{-}R296}$ (Fig. 4B). Therefore, we conclude that a functional PKR can specifically stimulate the expression of genes placed under the control of the HIV1 and HTLV-I

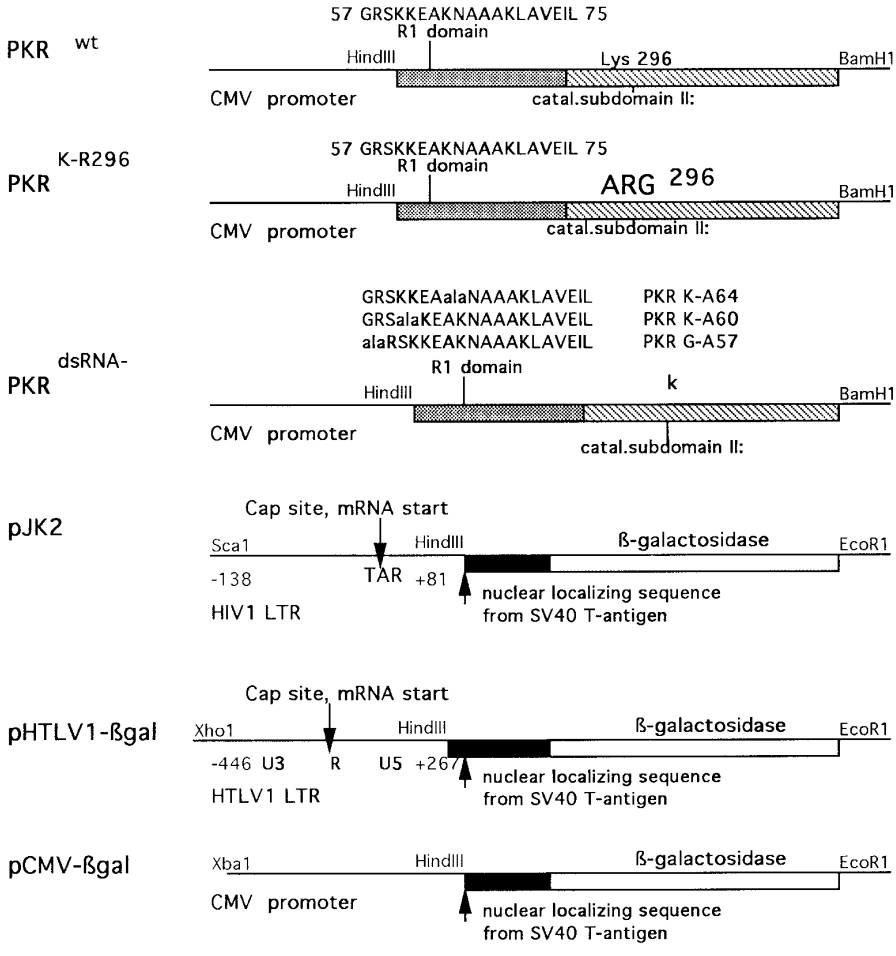

FIG. 1. Schematic representation of the different plasmids used for transfection. The plasmids pJK2 and pCMV-tat have been described previously (*24*). Briefly, pJK2 contains a truncated HIV1 LTR (−138 to +83 or *Sca*I to *Hin*dIII) followed by the complete β-galactosidase gene modified at its 5′ end to contain the nuclear targeting sequence of the SV40 T antigen. The plasmid pHTLV-I-βgal, corresponding to pJK2 but with the HTLV-I promoter instead of the HIV promoter, was constructed as follows: the plasmid HTLV-oligophX (*35*) containing the HTLV-I LTR of HTLV-I and the tax region (a gift from M. Nerenberg) was cut with *Xho*I and *Hin*dIII to yield the LTR from 1 to 713 bp. This fragment was ligated into the pEQJK plasmid cut with *Hin*dIII and *Sal*I. The plasmid pEQJK corresponds to the pJK2 plasmid but with a polylinker region 5′ of the nuclear-targeted β-galactosidase gene instead of the HIV promoter. To construct the plasmid pCMV-βgal, the CMV promoter was cut from the pCMV-tat plasmid with *Hin*dIII and *Xba*I and inserted between these sites in the polylinker region of plasmid pEQJK. The PKR^{wt} gene and its catalytically inactive mutant $PKR^{K\text{-}R296}$ form have been described previously (*4, 6*). In this study, the genes were expressed from the pcDNA/Amp vector instead of the pcDNA/neo vector. The pcDNA/neo vectors that express PKR mutants with altered dsRNA-binding properties have been recently described (*32*). Each of these mutants differs from PKR^{wt} by a single amino acid substitution Gly to Ala ($PKR^{G\text{-}A57}$) and Lys to Ala ($PKR^{K\text{-}A60}$, $PKR^{K\text{-}A64}$) in the carboxy-terminal region ($G_{57}RSK_{60}KEAK_{64}NAAKLAVEIL$) of the dsRNA-binding domain I of PKR. Such mutants do not bind ($PKR^{G\text{-}A57}$, $PKR^{K\text{-}A60}$), or bind poorly ($PKR^{K\text{-}A64}$), to dsRNA *in vitro* and cannot become activated (*32*). For PKR plasmids, the regulatory domain and the catalytic domains are represented in gray and hatched boxes, respectively. The catalytic subdomain II involved in the transfer of phosphate is inactivated by substitution of the lysine residue (Lys296 in PKR^{wt}) to arginine (Arg296 in $PKR^{K\text{-}R296}$). The carboxy-terminal part of dsRNA binding domain I (R1 domain) is indicated (residues 57 to 75) and the different substitutions to alanine residues leading to abrogation of dsRNA binding are indicated for the PKR^{dsRNA-} mutants. For the reporter plasmids, the nuclear-localizing sequence from the SV40 T antigen and the β-galactosidase sequence are represented by a black box and a white box, respectively.

LTRs, whereas the CMV promoter is not affected by this same regulatory mechanism.

The structure of the activator for PKR-mediated stimulation from the HTLV-I LTR remains to be determined. By analogy with the dsRNA TAR region of HIV1, which is known to bind PKR, it is possible that the 5′ untranslated sequence of the RNAs transcribed from the HTLV-I LTR also contains a dsRNA structure capable of binding and activating PKR. Indeed, recently, PKR has been reported to bind to, and be activated by, the HTLV-I Rex-response element in *in vitro* experiments (*33*). The Rex-response element is located in the U3/R of the HTLV-I LTR and presents a highly dsRNA secondary structure which is essential for rex regulation when expressed on the 3′ end of mRNAs (*34*). Since the CAP site is located within the Rex-response element, transcripts from the HTLV-I LTR also contain part of the Rex-response element, although deleted from its first 40 bases, involved in the formation

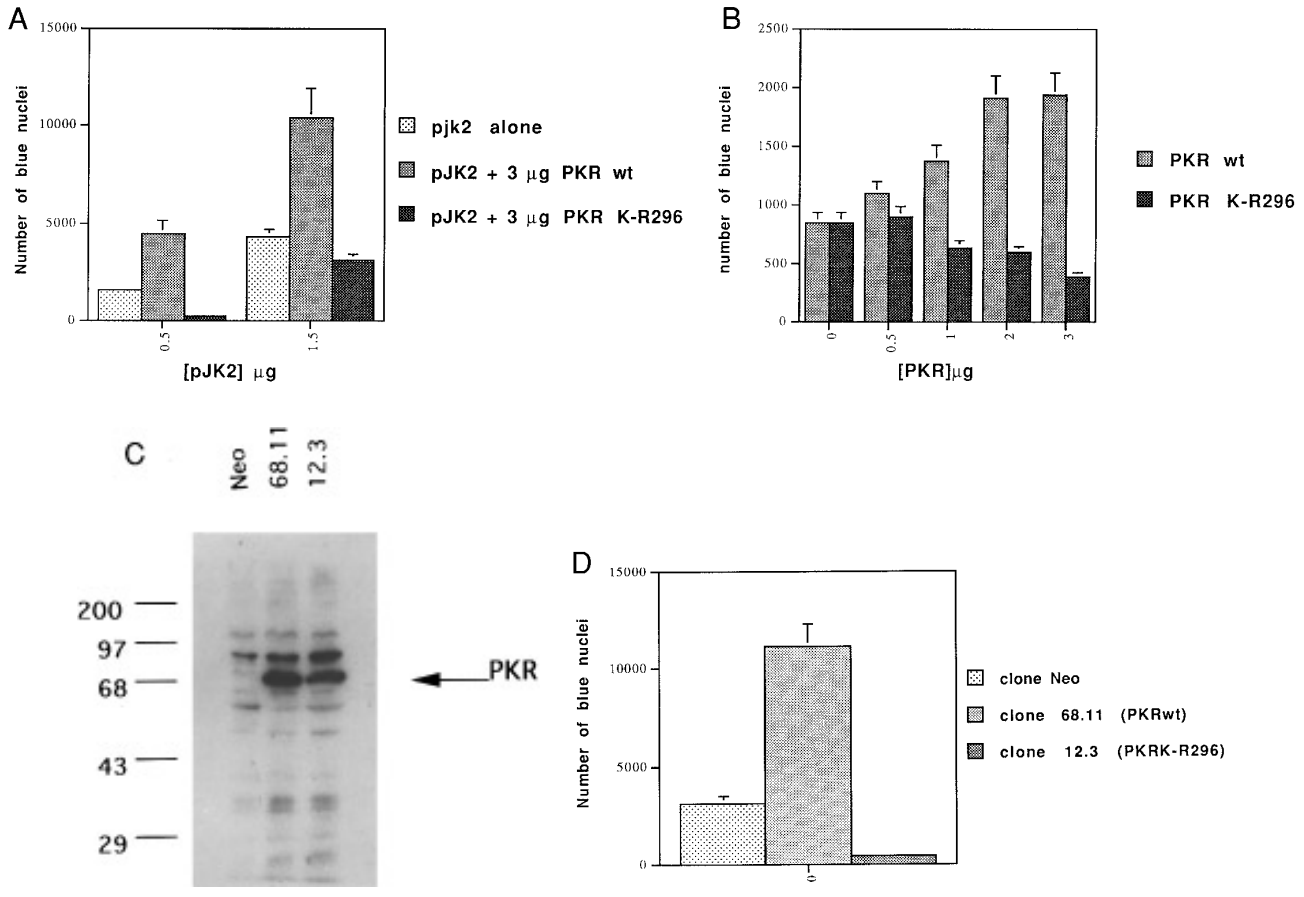

FIG. 2. Regulation of the expression of HIV1 LTR β-galactosidase by PKR. NIH/3T3 (Neo) cells were plated (3×10^5 cells) in 35-mm-diameter dishes in DMEM with 10% fetal calf serum, 18 to 24 hr before transfection. The medium was aspirated and replaced with fresh medium (4 ml per well) 3 hr before transfection. The desired amounts of plasmids were adjusted to the same final concentration of DNA by addition of pcDNA/Neo DNA, mixed in 250 μl of water before adding $CaCl_2$ to 250 m*M* and the resulting DNA/$CaCl_2$ solution was added dropwise to 250 μl of 2× HBS buffer (50 m*M* HEPES, 270 m*M* NaCl, 2.8 m*M* Na_2HPO_4, pH 7.11) while vortexing continuously. The DNA/calcium phosphate precipitate was added directly to the medium and the plates were incubated at 37° for 3 to 4 hr. The medium was removed and the cells were washed once with serum-free medium and overlaid for 2 min with 1 ml of 15% glycerol/1× HBS buffer. The cells were washed twice with complete medium and the plates were further incubated in complete medium for 48 hr at 37°. The expression of β-galactosidase was then measured by directly counting the cells expressing the enzyme: the medium was removed, the cells were washed once with phosphate-buffered saline (PBS) and fixed at room temperature with 1 ml of a solution of 1% formaldehyde–0.2% glutaraldehyde in PBS for 5 min. The cells were then washed three times with PBS and incubated for 50 min at 37° in 500 μl of a solution (in water) of 4 m*M* potassium ferrocyanide, 4 m*M* potassium ferricyanide, 2 m*M* $MgCl_2$, and 0.4 mg of X-Gal/ml. The reaction was stopped by removing the staining solution and washing the cells twice with PBS. Blue cells were counted under a microscope with a magnification of ×20 (*24*). In A, 3 μg of PKR^{wt} or $PKR^{K\text{-}R296}$ was cotransfected with 0.5 or 1.5 μg of pJK2. In B, the pJK2 concentration was kept constant (0.5 μg) and the concentration of the PKR plasmids varied (0.5, 1, 2, and 3 μg). (C) Constitutive expression of human PKR^{wt} and $PKR^{K\text{-}R296}$ in murine NIH/3T3 cell lines. The NIH/3T3 clones expressing either pcDNA/Neo alone (clone Neo) or pcDNA/Neo carrying the wt PKR (clone 68.11) or the $PKR^{K\text{-}R296}$ mutated in its subcatalytic subdomain II by a substitution from Lys to Arg (clone 12.3) have been described previously (*4*). Cells were grown in DMEM containing 10% fetal calf serum in the presence of 400 μg/ml G418 (geneticin; Gibco). Cells were washed and scraped in PBS, pelleted by centrifugation and proteins were extracted with 10 m*M* Tris–HCl, pH 7.6, 10 m*M* KCl, 1 m*M* EDTA, 7 m*M* 2-mercaptoethanol, 0.5% Triton X-100, 0.2 m*M* phenylmethylsulfonylfluoride (PMSF), 10 μg/ml aprotinin, 10 μg/ml leupeptin. After 10 min at 4°, the extracts were diluted twice with B.I buffer (20 m*M* Tris–HCl, pH 7.6, 50 m*M* KCl, 400 m*M* NaCl, 1 m*M* EDTA, 1% Triton X-100, 5 m*M* 2-mercaptoethanol, 0.2 m*M* PMSF, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 20% glycerol) and centrifuged at 12,000 *g,* and the supernatants were aliquoted and frozen at −80°. For immunoblot analysis, the cytoplasmic protein extracts, corresponding to 2×10^6 cells, were separated by SDS–PAGE in 12.5% polyacrylamide gels, transferred to Immobilon-P membranes (Millipore), and incubated with polyclonal antibodies to PKR (*26*) and anti-mouse immunoglobulin coupled to horseradish peroxidase (ECL; Amersham). PKR was visualized after incubation with ECL reagents and exposure to X-ray film (Kodak). (D) Two micrograms of pJK2 was transfected into the different clones, Neo, 68.11, or 12.3.

of its two major stems. It is possible that, as such, the Rex-response element refolds in a different secondary structure sufficient to allow the *in vivo* activation of PKR that we have observed. On the other hand, the fact that the three PKR dsRNA binding mutants, $PKR^{G\text{-}A57}$, $PKR^{K\text{-}A60}$, and $PKR^{K\text{-}A64}$, were still partially functional in stimulating the LTR from HTLV-I raises questions about the mechanism of activation. Whatever the nature of the activator for HTLV-I, it must either interact with amino acid residues in the dsRNA binding domain I of PKR other than glycine57,

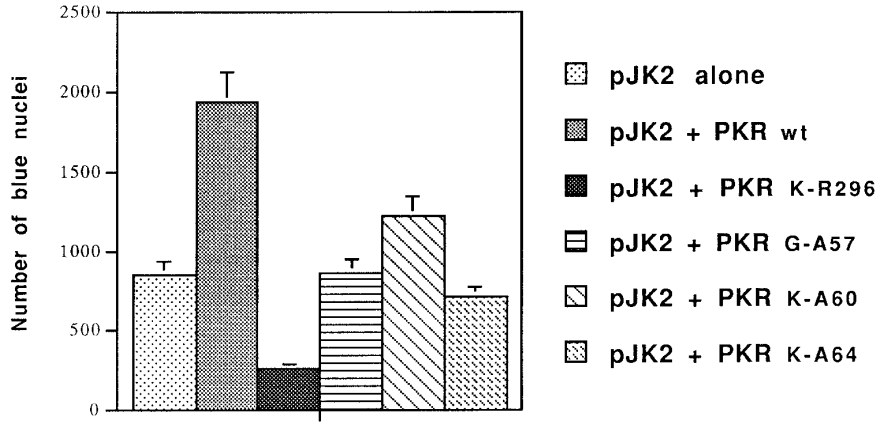

FIG. 3. The stimulation of HIV1 LTR by PKR depends on a functional dsRNA binding region in the regulatory domain of the enzyme. The murine NIH/3T3 (Neo) cells were transfected with 0.5 μg of pJK2 in the presence of 3 μg of PKR^{wt}, the catalytically inactive mutant $PKR^{K\text{-}R296}$, or each of the three dsRNA− mutants: $PKR^{G\text{-}A57}$, $PKR^{K\text{-}A60}$, and $PKR^{K\text{-}A64}$. Forty-eight hours after transfection, the expression of β-galactosidase was assayed as described under Fig. 2.

lysine60, or lysine64 or, alternatively, it may interact with activation domains of PKR other than domain I. In this regard, it has been recently reported that a dsRNA binding mutant of PKR expressed *in vivo* from a vaccinia virus vector could be activated on domains other than those required for binding dsRNA (*30*).

Here, we have shown that direct expression of PKR affects the expression of a reporter gene under the con-

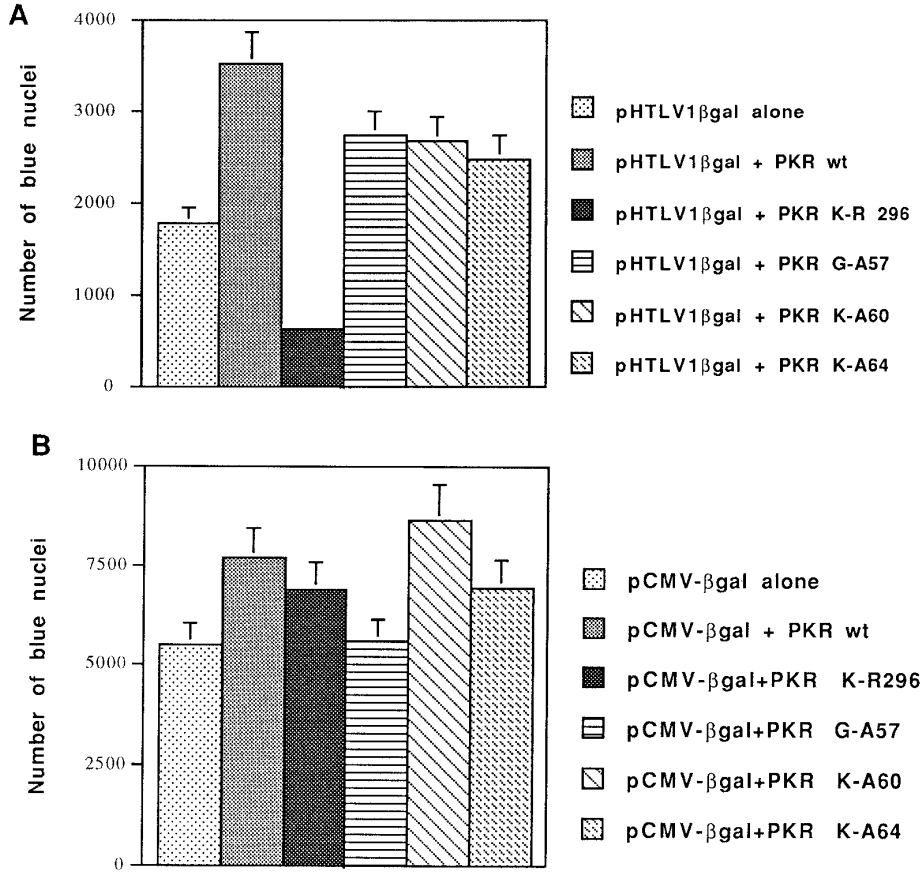

FIG. 4. PKR specifically stimulates the expression of genes placed under the control of the HTLV-I LTR but not under the control of the CMV immediate-early promoter. The murine NIH/3T3 (Neo) cells were transfected with (A) 0.5 μg of pHTLV-I–β-galactosidase or with (B) 0.5 μg of pCMV-β galactosidase in the presence of 3 μg of PKR^{wt}, the catalytically inactive mutant $PKR^{K\text{-}R296}$, or each of the three dsRNA− mutants: $PKR^{G\text{-}A57}$, $PKR^{K\text{-}A60}$, and $PKR^{K\text{-}A64}$. Forty-eight hours after transfection, the expression of β-galactosidase was assayed as described under Fig. 2.

trol of the LTR from HIV1. In the presence of the wild-type PKR, expression from the LTR was stimulated. Expression was unchanged in the presence of PKR^{dsRNA-} mutants and inhibited when cotransfection was carried out with the catalytically inactive mutant $PKR^{K\text{-}R296}$. The data with PKR^{wt} and with PKR^{dsRNA-} mutants, taken together, strongly suggest that PKR has been activated *in vivo,* most probably by the TAR region of the LTR of HIV1 since this region has previously been shown to activate PKR (*20, 21*). It should be emphasized, however, that our *in vivo* assay does not allow one to test whether PKR activation was dependent on TAR and thus we cannot exclude a possible contribution from other potential dsRNA structures in the HIV1 LTR distinct from TAR.

The data for cotransfection with PKR^{wt} are also consistent with experiments reported previously showing stimulation of expression of a reporter gene cotransfected with a TAR-expressing vector (*22, 23*). In this latter case, stimulation was explained as resulting from the sequestration of PKR by high inhibitory levels of TAR RNA and the consequential failure of PKR to regulate the initiation of protein synthesis through eIF2 phosphorylation. Therefore, the stimulation of expression should occur independently of the nature — wild type or catalytically inactive mutant — of the sequestered PKR. Since our data showed a net inhibition of expression in the presence of the catalytically inactive $PKR^{K\text{-}R296}$, this indicates that functional PKR was directly involved in the stimulation of expression of the reporter gene, placed under the control of the HIV1 LTR.

What are the events following PKR activation that lead to stimulation of the HIV1 LTR? The best known substrate for PKR is the initiation factor eIF2. Phosphorylation of eIF2 by PKR negatively affects protein synthesis. Although it is possible that some eIF2 might be phosphorylated as a consequence of PKR activation, it is unlikely that eIF2 plays a major role here, since its PKR^{wt}-mediated phosphorylation would lead to an inhibition of translation, hence an inhibition of the expression of the reporter gene, instead of the stimulation that we observed. Moreover, prevention of the phosphorylation of eIF2 in presence of the mutant $PKR^{K\text{-}R296}$ would yield levels of expression comparable to the controls rather than the inhibition of expression that was observed. We therefore postulate the existence of another substrate which can be phosphorylated by the activated PKR. Once phosphorylated, this substrate would augment expression from the HIV1 LTR, whereas the prevention of its phosphorylation would lead to inhibition of expression. PKR was recently shown to phosphorylate IκB, the cytoplasmic inhibitor of NF-κB (*7*). When activated by dsRNA *in vitro* or *in vivo,* PKR was shown to be directly implicated in the poly(I)–poly(C) induction of NF-κB-dependent reporter constructs, through NF-κB activation (*7, 8*). At present, there is no evidence that PKR-mediated activation of the HIV1 LTR, in our *in vivo* experiments, involves IκB phosphorylation. Attempts to detect NF-κB activation by gel-shift assay were inconclusive. Cell extracts from doubly transfected NIH/3T3 cells did not contain any detectable NF-κB activity, most probably due to lack of sensitivity in the experiment since only a small number of cells are expressing both PKR and the HIV1 LTR promoter (data not shown). In the clone constitutively expressing PKR^{wt}, NF-κB was found to be active constitutively, masking the observation of any possible increase after transfection with the HIV LTR plasmid (data not shown). The development, however, of an expression system based on conditional expression of PKR should allow this question to be addressed directly. There is also a possibility that the substrate involved in the stimulation of genes expressed from the HIV1 LTR acts at a posttranscriptional level, favoring, for example, the stabilization of the new transcripts or their ability to be correctly translated.

Thus PKR, in addition to having the ability to regulate gene expression at the translational level through eIF2 phosphorylation, can also be involved in the stimulation of gene expression. Dual regulation of both translation and transcription by PKR in virus-infected cells is likely to be important in determining the activation state of HIV1 and HTLV-I genomes and may influence the progression from latent to productive infections.

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REFERENCES

1. Hovanessian, A. G., and Galabru, J., *Eur. J. Biochem.* 167, 467–473 (1987).
2. Hershey., *Annu. Rev. Biochem.* 60, 717–755 (1991).
3. Hovanessian, A. G., *Semin. Virol.* 4, 237–245 (1993).
4. Meurs, E. F., Watanabe, Y., Kadereit, S., Barber, G. N., Katze, M. G., Chong, K., Williams, B. R. G., and Hovanessian, A. G., *J. Virol.* 66, 5805–5814 (1992).
5. Koromilas, A. E., Roy, S., Barber, G. N., Katze, M. G., and Sonnenberg, N., *Science* 257, 1685–1689 (1992).
6. Meurs, E. F., Galabru, J., Barber, G. N., Katze, M. G., and Hovanessian, A. G., *Proc. Natl. Acad. Sci. USA* 90, 232–236 (1993).
7. Kumar, A., Haque, J., Lacoste, J., Hiscott, J., and Williams, B. R. G., *Proc. Natl. Acad. Sci. USA* 91, 6288–6292 (1994).
8. Maran, A., Maitra, R. K., Kumar, A., Dong, B., Xiao, W., Li, G., Williams, B. R. G., Torrence, P. F., and Silverman, R. H., *Science* 265, 789–792 (1994).
9. Katze, M. G., De Corato, Safer, B., Galabru, J., and Hovanessian, A. G., *EMBO J.* 6, 689–697 (1987).
10. Clarke, P. A., Schwemmle, M., Schickingger, J., Hilse, K., and Clemens, M. J., *Nucleic Acids Res.* 19, 243–248 (1991).
11. Chang, H. W., Watson, J. C., and Jacobs, B. L., *Proc. Natl. Acad. Sci. USA* 89, 4825–4829 (1992).
12. Imani, F., and Jacobs, B. L., *Proc. Natl. Acad. Sci. USA* 85, 7887–7891 (1988).

13. Davies, M., Chang, H-W., Jacobs, B. L., and Kaufman, R. J., *J. Virol.* 67, 1688–1692 (1993).
14. Black, T. L., *et al., J. Virol.* 63, 2244–2251 (1989).
15. Lee, T. G., Tang, N., Thompson, S., Miller, J., and Katze, M. G., *Mol. Cell. Biol.* 14, 2331–2342 (1994).
16. Dubois, M. F., and Hovanessian, A. G., *Virology* 179, 591–598 (1990).
17. Roy, S., Agy, M., Hovanessian, A. G., Sonnenberg, N., and Katze, M. G., *J. Virol.* 65, 632–640 (1991).
18. Parkin, N. T., Cohen, E. A., Darveau, A., Rosen, C., Haseltine, W., and Sonnenberg, N., *EMBO J.* 7, 2831–2837 (1988).
19. Edery, I., Petryshyn, R., and Sonenberg, N., *Cell* 56, 303–312 (1989).
20. Sengupta, D. N., and Silverman, R. H., *Nucleic Acids Res.* 17, 969–978 (1989).
21. Maitra, R. K., McMillan, N. A. J., Desai, S., McSwiggen, J., Hovanessian, A. G., Sen, G., Williams, B. R. G., and Silverman, R. H., *Virology* 204, 823–827 (1994).
22. Gunnery, S., Rice, A. P., Robertson, H. D., and Mathews, M. B., *Proc. Natl. Acad. Sci. USA* 87, 8687–8691 (1990).
23. Gunnery, S., Green, S. R., and Mathews, M. B., *Proc. Natl. Acad. Sci. USA* 89, 11557–11561 (1992).
24. Kimpton, J., and Emerman, M., *J. Virol.* 66, 2232–2239 (1992).
25. Hovanessian, A. G., Meurs, E., and Montagnier, L., *J. Interferon Res.* 1, 170–190 (1981).
26. Meurs, E., Chong, K., Galabru, J., Thomas, S. B., Kerr, I. M., Williams, B. R. G., and Hovanessian, A. G., *Cell* 62, 379–390 (1990).
27. Feng, G. S., Chong, K., Kumar, A., and Williams, B. R. G., *Proc. Natl. Acad. Sci. USA* 89, 5447–5451 (1992).
28. Green, S. R., Manche, L., and Mathews, M. B., *Mol. Cell. Biol.* 15, 358–364 (1995).
29. Green, S. R., and Matthews, M. B., *Genes Dev.* 6, 2478–2490.
30. Lee, S. B., Green, S. R., Mathews, M. B., and Esteban, M., *Proc. Natl. Acad. Sci. USA* 91, 10551–10555 (1994).
31. Chong, K. L., Schappert, K., Meurs, E., Feng, L., Donahue, T. F., Friesen, J. D., Hovanessian, A. G., and Williams, B. R. G., *EMBO J.* 11, 1553–1562 (1992).
32. McMillan, N. A. J., Carpick, B. W., Hollis, B., Toone, M. W., Zamanian-Daryoush, M., and Williams, B. R. G., *J. Biol. Chem.* 270, 2601–2606 (1995).
33. Mordechai, E., Ning Kon, Henderson, E. E., and Suhadolnik, R. J., *Virology* 206, 913–922 (1995).
34. Toyoshima, H., Itoh, M., Inoue, J. I., Seiki, M., Takaku, F., and Yoshida, M., *J. Virol.* 64, 2825–2832 (1990).
35. Nerenberg, M., Hinrichs, S. H., Reynolds, P. K., Khouty, G., and Jay, G., *Science* 237, 1324–1329 (1987).