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The antiviral enzymes PKR and RNase L suppress gene expression from viral and non-viral based vectors

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ABSTRACT

Expression of transfected genes is shown to be suppressed by two intracellular enzymes, RNase L and protein kinase PKR, which function in interferon-treated cells to restrict viral replication. RNase $L^{-/-}$ or $PKR^{-/-}$ murine embryonic fibroblasts produced enhanced levels of protein from transfected genes compared with wild-type cells. Increased expression of exogenous genes in RNase $L^{-/-}$ cells correlated with elevated levels of mRNA and thus appeared to be due to enhanced mRNA stability. Plasmid encoding adenovirus VA RNAs was able to further enhance accumulation of the exogenous gene transcript and protein, even in cells lacking PKR. In contrast to the increased expression of transfected genes in cells lacking RNase L or PKR, expression of endogenous host genes was unaffected by the absence of these enzymes. In addition, a dominant-negative PKR mutant improved expression from a conventional plasmid vector and from a Semliki Forest virus derived, self-replicating vector. These results indicate that viral infections and transfections produce similar stress responses in mammalian cells and suggest strategies for selectively increasing expression of exogenous genes.

INTRODUCTION

Nucleic acid delivery into mammalian cells by viruses and transfections results in similar forms of cellular stress that limits gene expression (Fig. 1). Double-stranded RNA (dsRNA) produced by viruses induces type I interferons (IFNs) but also directly activates two types of IFN-induced proteins, PKR (dsRNA-dependent protein kinase) and 2′,5′-oligo A (2-5A) synthetases (1). In addition, recombinant DNA vectors may contain cryptic promoters that generate antisense strands of RNA which anneal with the mRNA to form dsRNA (2) (Fig. 1). Activated PKR blocks protein synthesis by phosphorylating the α subunit of eukaryotic initiation factor eIF2, thereby sequestering the recycling factor, eIF2B/GEF (1). On the other hand, 2-5A synthetases produce short, 2′,5′-linked oligoadenylates which activate RNase L, a single-stranded specific endoribonuclease that degrades mRNA and rRNA (1).

The control of exogenous gene expression by cellular enzymes is a poorly understood phenomenon. Previous studies have suggested that PKR limits protein synthesis from transfected genes (3–7). A Ser(51)→Ala mutant eIF2α, preventing phosphorylation at this critical position, selectively stimulated translation of plasmid-derived transcripts, whereas a Ser(51)→Asp mutant eIF2α which mimics a phosphorylated residue, inhibited protein synthesis from such transcripts (4). The assignment of such effects to PKR is complicated by the existence of other mammalian eIF-2α kinases, such as PERK (PKR-like endoplasmic reticulum kinase) and HRI (heme regulated inhibitor) (8–10). Many types of viruses promote their replication with inhibitors of PKR (1) and both viral (adenovirus VAI RNA) and chemical (2-aminopurine) inhibitors of PKR have been shown to enhance the translation efficiency of exogenously expressed mRNA in mammalian cells (3,4,7). The finding of selectively enhanced expression of exogenous genes by 2-aminopurine treatment extends to stably transfected cells (7). In two studies expression of VAI RNA stabilized ribosome-associated RNAs, which could lead to enhanced levels of protein synthesis independent of any effect on PKR (11,12). An additional consideration is that liposomal reagents often used to facilitate DNA uptake can by themselves induce IFN-β synthesis which will lead to elevated levels of PKR, 2-5A-synthetase and RNase L (13).

Therefore, to extend these studies to the 2-5A system and to obtain definitive evidence for effects of PKR on exogenous gene expression, we performed studies on cell lines generated from wild-type, RNase $L^{-/-}$ or $PKR^{-/-}$ mouse embryos (14,15). We also investigated the effect of co-expressing a dominant-negative PKR on gene expression from conventional expression plasmids and from a viral-based, self-replicating vector (16). Results show that PKR and RNase L substantially limit expression of exogenous genes in mammalian cells.

MATERIALS AND METHODS

Cell culture and transient transfection of plasmids

Murine embryonic fibroblasts (MEFs) were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS). To minimize the

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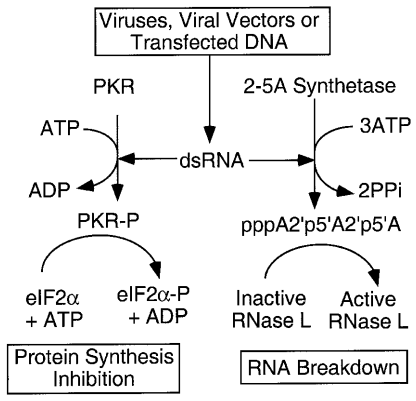

Figure 1. Role of the PKR and 2-5A/RNase L systems in controlling expression of genes encoded by viruses, viral vectors and transfected DNA.

possibility that clonal variability affected results, all cells used consisted of pools of post-crisis MEFs (14,17). MEFs were seeded 16 h before transfections at a density of 1×10^6 cells per 100 mm diameter dish or at 1×10^5 per well in 6-well plates. Cells were transfected using lipofectamine-PLUS transfection reagent (Gibco BRL) according to manufacturer's protocol. Plasmids used were pSV-β-galactosidase containing β-galactosidase cDNA (Promega) and pRL-SV40 luciferase or pRL-CMV luciferase, vectors containing *Renilla* luciferase (RL) cDNA under control of an SV40 early promoter or a CMV promoter (Promega). Human CD4 cDNA was fused to the AU-rich RNA destablizing element (ARE) from the GM-CSF 3′-UTR or to a mutant version of the ARE containing Gs and Cs in place of As and Ts in plasmid pcDNA in a method which included PCR (18) (S. Naik and R.H. Silverman, unpublished data). The GM-CSF ARE and the GC mutant ARE were gifts from G. Shaw (Genetics Institute, Cambridge, MA). The rat apobec cDNA in pCR3.1/APOBEC was under control of a CMV promoter (a gift of D. Driscoll, Cleveland, OH). In some experiments, pSV-β-galactosidase was co-transfected with pRcCMV/PKRm encoding a dominant-negative mutant PKR, lysine→arginine at position 296 (16), or pAdVAntage containing base pairs 9831–11 555 of the adenovirus type 2 genome encoding the virus-associated RNA genes VAI and VAII (Promega). The cells were incubated with lipofectaminePLUS/DNA mixture for 4 h at 37°C, washed with PBS and incubated with DMEM plus 10% FBS for 18 h.

Luciferase and β-galactosidase assays

Luciferase and β-galactosidase activity assays were performed as described (Promega). Cell pellets were solubilized with 0.1 M NaOH and neutralized before determining protein concentrations by the Bradford method (Bio-Rad). Negative controls were done on untransfected cells. Luciferase and β-galactosidase levels were normalized for protein concentrations.

RNA extraction and northern blot analysis

Total cellular RNA was prepared from transfected cells with RNAzol reagent according manufacturer's protocol (CINNA/BIOTEX). RNAs were separated in 1.2% agarose–formaldehyde gel, and transferred into Nylon transfer membrane (Amersham) for 18–20 h. The membranes were hybridized with [^{32}P]dCTP-labeled probe prepared by random priming using the 'rediprime' DNA labeling system (Amersham).

Assay for protein synthesis

Total cellular protein synthesis was measured by trichloroacetic acid (TCA) precipitation (19). At 18 h after transfections, cells in a 6-well plate (1×10^5 cells/well) were incubated with 100 μCi/ml of ^{35}S-L-methionine in serum-free medium for 1 h at 37°C. Experiments were performed in triplicate and the mean values are reported. Protein synthesis was monitored in 10% SDS–polyacrylamide gels. After labeling with 100 μCi/ml ^{35}S-L-methionine the supernatants were discarded, cells were washed with PBS three times, gel sample buffer was added, proteins were separated in SDS–10% polyacrylamide gels and visualized in autoradiograms of the dried gels.

Western blots

After transfections, cells were washed in PBS and lysed in NP-40 lysis buffer (20). Protein (100 μg per lane) was loaded onto SDS–10% polyacrylamide gels. After electrophoresis, proteins were transferred to Immobilion-p membrane and immunodetection was done using monoclonal antibody against β-galactosidase (Promega). Anti-mouse IgG tagged with horseradish peroxidase (Gibco BRL) was used as a secondary antibody. Detection was done using ECL western blotting detection system (Amersham). The proteins in the blots were also stained with 0.1% amido black.

Transfections with the self-replicating viral-based vector, pCMV-REP-*LacZ*

The plasmid, pCMV-REP-*LacZ* containing a CMV promoter driving expression of an alpha virus replicase/β-galactosidase construct was made as follows. The human CMV immediate-early enhancer/promoter region was amplified by PCR from the pCR1 plasmid (Promega, Madison, WI). *Sph*I sites were added during PCR amplification. The PCR fragment was then cloned into the plasmid pSFV-*LacZ* which contains the Semliki Forest Virus replicon (Life Technologies, Grand Island, NY). To facilitate RNA transport, a chimeric intron was introduced at the *Xma*I site that is 3′ to the *LacZ* gene. This chimeric intron, with added *Xma*I sites, was obtained by a PCR amplification from the pCI plasmid (Promega). BHK-21 and Cos-7 cells were grown in DMEM supplemented with 10% FBS. BHK-21 cells were seeded at 1.2×10^6 cells and Cos-7 cells were seeded at 0.75×10^6 cells per 60 mm plate for 16 h prior to transfections. BHK-21 and Cos-7 cells were transfected using lipofectaminePLUS as described above with pCMV-REP-*LacZ* co-transfected with pRcCMV/PKRm (expressing the dominant-negative PKR mutant) (16), pRcCMV/PKR expressing wild-type human PKR cDNA, and pRcCMV (the 'empty' vector control) at equimolar amounts.

RESULTS

Exogenous genes are expressed to high levels in RNase L$^{-/-}$ and PKR$^{-/-}$ cells

To determine the effects of PKR and RNase L on the regulation of exogenous gene expression, RNase L$^{-/-}$, PKR$^{-/-}$ and wild-type

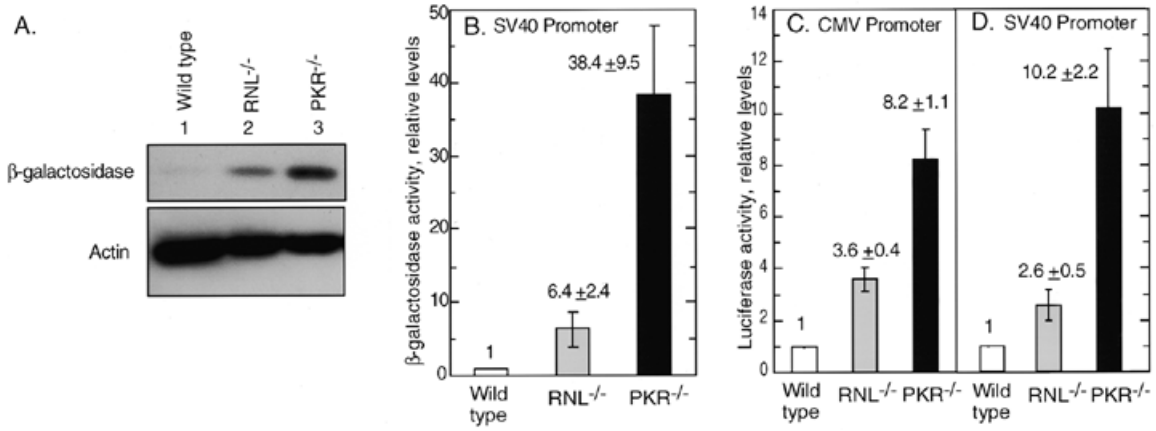

Figure 2. Exogenous genes are expressed to high levels in RNase $L^{-/-}$ ($RNL^{-/-}$) and $PKR^{-/-}$ cells. (**A**) Western blot assay from cells transfected with pSV-β-galactosidase and probed with antibody to β-galactosidase or actin. (**B**) β-Galactosidase assays from cells transfected with pSV-β-galactosidase (4 μg) (data are averages of three independent experiments, each performed in duplicate). Luciferase assays after transfections with (**C**) pRL-SV40 luciferase (1 μg) or (**D**) pRL-CMV luciferase (1 μg). Data are averages of six independent experiments; each performed in duplicate.

MEF cell lines were transiently transfected with plasmid encoding β-galactosidase (14,15). Western blots probed with antibody indicated that β-galactosidase protein levels were greatly elevated in both types of knockout cells compared with the wild-type cells (Fig. 2A). Accordingly, β-galactosidase enzymatic activity was increased 6- and 38-fold in the RNase $L^{-/-}$ and $PKR^{-/-}$ cells, respectively (Fig. 2B). While the presence of PKR had a greater effect than RNase L, it was clear that both proteins significantly suppressed production of β-galactosidase. These results were extended to another transgene and promoter by transiently transfecting cells with luciferase cDNA under control of either a CMV or an SV40 promoter. Compared to wild-type cells, luciferase activity was 3.6- and 2.6-fold higher in the RNase $L^{-/-}$ cells and 8.2- and 10.2-fold higher in the $PKR^{-/-}$ cells when expressed from a CMV or SV40 promoter, respectively (Fig. 2C and D). Therefore, different transgenes under control of different promoters were expressed at much higher levels in cells lacking RNase L or PKR.

The combined effects of PKR and RNase L were investigated using MEFs derived from double knockout mice (17). Post-crisis $PKR^{-/-}$/RNase $L^{-/-}$ MEFs were transfected with pSV-β-galactosidase. Curiously, exogenous gene expression in the double knockout cells was not higher than in the PKR knockout cells (6.4-, 12.5- and 38.4-fold increases in β-galactosidase activity in the RNase $L^{-/-}$, $PKR^{-/-}$/RNase $L^{-/-}$ and $PKR^{-/-}$ cells, respectively, relative to levels in wild-type cells). Whether this situation will apply to other cell types derived from the double knockout mice, or is unique to MEFs, is unknown.

Exogenous gene transcripts accumulate to high levels in RNase $L^{-/-}$ cells

To determine the effect of RNase L on steady-state levels of several different transcripts, northern blot assays were performed after transient transfections. The absence of RNase L resulted in elevated levels of transcripts from different genes under the control of various promoters (Fig. 3). For example, accumulation of apobec mRNA, encoding an RNA editing enzyme, transcribed from a transfected cDNA driven with a CMV promoter was 7-fold higher in the RNase $L^{-/-}$ cells than in wild-type cells (Fig. 3A). Similarly, β-galactosidase cDNA driven by an SV40 promoter expressed 11.5-fold more mRNA in RNase $L^{-/-}$ cells than in wild-type cells (Fig. 3B). To examine if the RNase L effect could be extended to a highly unstable mRNA, CD4 cDNA was fused to either a 51 nt ARE from the GM-CSF 3′-UTR and to a stabilized, mutated version of the ARE containing Gs and Cs (18). Prior experiments performed in human HT1080 cells showed the stabilized CD4-GC transcript accumulated to 6-fold higher levels than the destabilized CD4-AU mRNA (data not shown). In contrast, both the stabilized (CD4-GC) and destabilized (CD4-AU) transcripts accumulated to similar high levels in the RNase $L^{-/-}$ cells (3.7- and 5.2-fold increases compared to wild-type cells; Fig. 3C and D, respectively). Endogeneous 28S rRNA and GAPDH mRNA was present at constant levels in these experiments, despite the fact that rRNA can be cleaved by RNase L in intact ribosomes (21). These results suggest that RNase L inhibits gene expression by selectively degrading transcripts generated from several different exogenous genes.

PKR independent effects of adenovirus VA RNAs

To determine if adenovirus VA RNAs could further increase protein expression, cells were co-transfected with pAdVAntage (Promega) encoding adenovirus VAI and II RNAs. β-galactosidase activity was enhanced by up to 21-fold by co-transfecting pAdVAntage in the wild-type and RNase $L^{-/-}$ cells, respectively (Fig. 4). Surprisingly, the VA RNAs enhanced β-galactosidase activity in $PKR^{-/-}$ cells by 95-fold (Fig. 4). Therefore, the stimulatory effect of VAI RNA is not only observed, but it is enhanced in the absence of PKR. When cells were pulse-labeled for 1 h with ^{35}S-L-methionine 18 h after transfection with pAdVAntage, the global pattern of protein synthesis appeared unchanged, while the total rate of protein synthesis actually decreased (Fig. 5A and B). Co-transfections with pAdVAntage caused β-galactosidase protein levels to increase without increasing endogenous actin levels (Fig. 6A). Similar results were obtained in the RNase $L^{-/-}$ cells (data not shown).

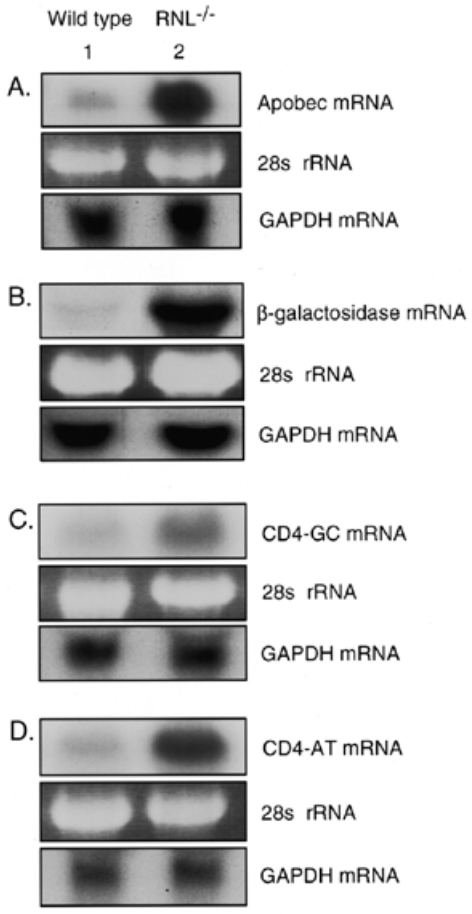

Figure 3. RNA from transfected genes accumulates to elevated levels in RNase $L^{-/-}$ ($RNL^{-/-}$) cells. Northern blots of (**A**) apobec and (**B**) β-galactosidase, (**C**) CD4-GC and (**D**) CD4-AT mRNAs and of endogenous GAPDH mRNA (lower panels) in wild-type and RNase $L^{-/-}$ ($RNL^{-/-}$) cells in comparison to ethidium bromide stained 28S rRNA (middle panels). Total cellular RNA (30 µg per lane) from cells transfected for 18 h with 2 µg (A), (C) and (D) or 4 µg (B) of plasmid.

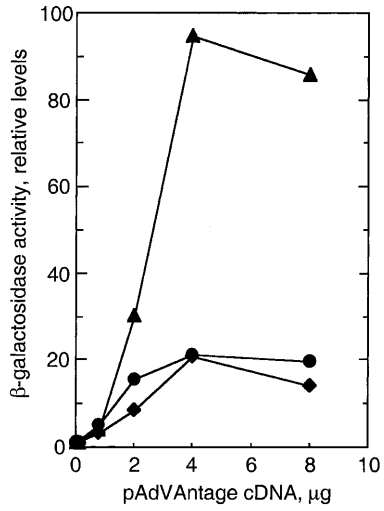

Figure 4. Adenovirus VA RNAs enhance exogenous gene expression in wild-type (circles), RNase $L^{-/-}$ (diamonds) and $PKR^{-/-}$ cells (triangles). Cells were transiently co-transfected with pSV-β-galactosidase (2 µg) and different amounts of pAdVAntage (as indicated).

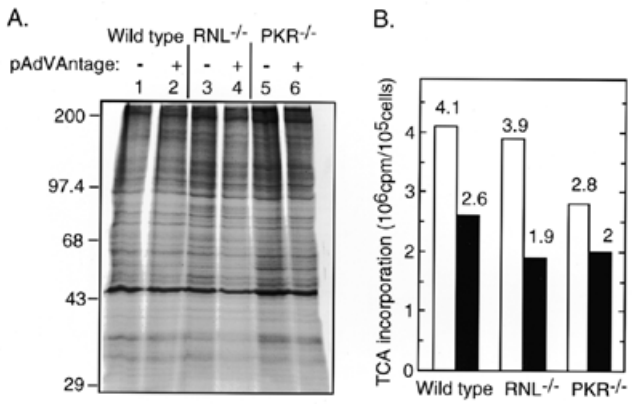

Figure 5. Expression of adenovirus VA RNAs does not enhance protein synthesis in general. (**A**) An autoradiogram of ^{35}S-L-methionine pulse-labeled proteins in the presence or absence of pAdVAntage (4 µg) (as indicated). (**B**) Incorporation of ^{35}S-L-methionine into protein in pulse-labeled cells in the absence (open bars) or presence (closed bars) of pAdVAntage.

Previously, it was reported that VAI RNA led to increased RNA stability (11,12). Similarly, we observed co-transfection with pAdVAntage increased β-galactosidase mRNA amounts to different levels in the different cell types in the order wild-type < RNase $L^{-/-}$ < $PKR^{-/-}$ (Fig. 7). It is this effect on RNA accumulation, and not inhibition of PKR, which is likely to be principally responsible for the stimulation of exogenous gene expression in response to VAI RNA.

Expression of a self-replicating viral vector is enhanced with a dominant-negative PKR mutant

To determine if suppressing PKR function could mimic effects of the PKR gene knockout, we expressed a dominant-negative PKR mutant (PKRm; Lys→Arg at position 296) that lacks kinase activity (16). Co-transfections of PKRm cDNA enhanced gene expression in the wild-type (5-fold) and RNase L null cells (20-fold), but it did not enhance expression in the $PKR^{-/-}$ cells (Fig. 8). A control plasmid, pRcCMV, had little effect on expression of β-galactosidase in the different cell types.

Finally, we investigated whether the enhancement of protein expression observed in the absence of PKR with conventional expression vectors was also seen with an alpha viral-based self-replicating vector (22,23). The vector we employed, pCMV-REP-*LacZ*, produces requisite dsRNA intermediates during its replication (24), and thus has the potential to activate PKR and 2-5A synthetase. BHK-21 and Cos-7 cells were used because they were readily transfected with the large, 15.1 kb pCMV-REP-*LacZ* plasmid. Co-transfection of pCMV-REP-*LacZ*,

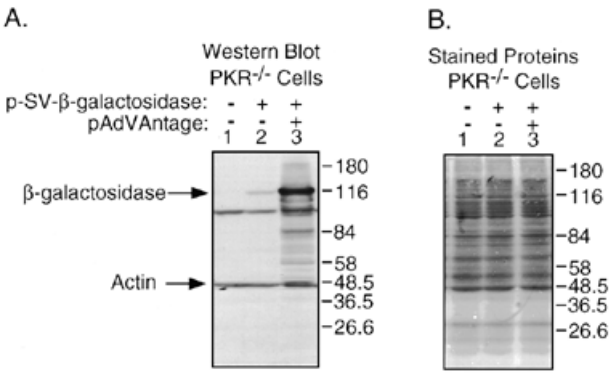

Figure 6. Selective enhancement of β-galactosidase protein accumulation by VA RNAs. (**A**) Western blot probed with antibody against β-galactosidase and actin. Lane 1, no transfection; lane 2, transfection with p-SV-β-galactosidase (2 µg); lane 3, co-transfection with p-SV-β-galactosidase (2 µg) and pAdVAntage (4 µg). (**B**) Proteins stained with amido black.

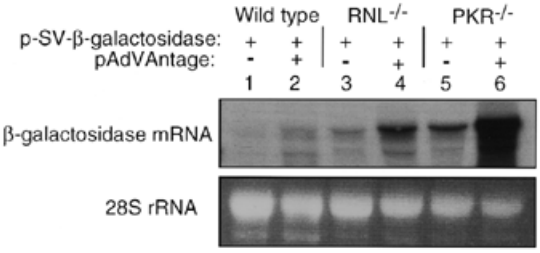

Figure 7. Expression of VA RNAs enhances levels of β-galactosidase mRNA after transfections with p-SV-β-galactosidase (2 µg) and pAdVAntage (4 µg) (upper panel). Ethidium bromide staining of 28S rRNA (lower panel).

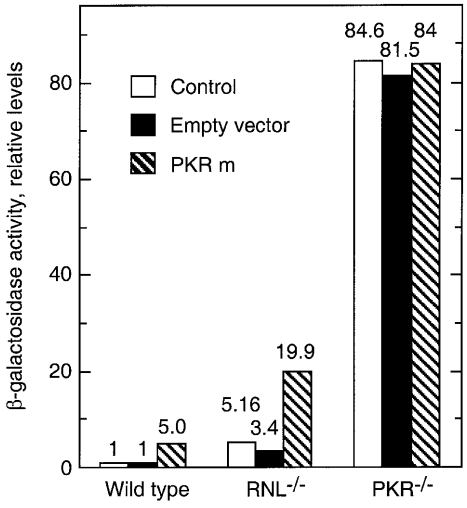

Figure 8. A dominant-negative PKR mutant, PKRm, enhances expression of β-galactosidase in wild-type and RNase L$^{-/-}$ (RNL$^{-/-}$) cells but not in PKR$^{-/-}$. Cells were transiently transfected with pSV-β-galactosidase (4 µg) alone (control), or with pRcCMV (4 µg) (empty vector), or with pRcCMV/PKRm (4 µg) (expressing PKRm). β-Galactosidase activity at 18 h represents fold induction compared with wild-type cell levels. Values are averages from two experiments each performed in duplicate.

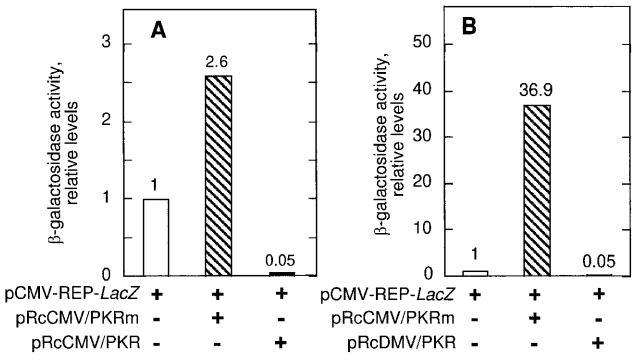

Figure 9. Inhibition of PKR increases β-galactosidase expression from a Semliki forest virus based vaccine vector, pCMV-REP-*LacZ*. (**A**) BHK-21 or (**B**) Cos-7 cells were co-transfected with pCMV-REP-*LacZ* [1.5 µg in (A) and 1.0 µg in (B)] and 0.75 µg of either empty vector (pRcCMV) or the same vector encoding dominant-negative PKR (PKRm) or wild-type PKR. After 20 h cells were harvested and assayed for β-galactosidase activity. Average values for two independent experiments are shown.

with a dominant-negative form of PKR (pRcCMV/PKRm) increased the amount of β-galactosidase activity produced from the viral expression construct in BHK-21 cells (2.6-fold) and Cos-7 cells (36.9-fold) relative to co-transfection with the empty pRcCMV vector (Fig. 9A and B). In contrast, co-transfection of wild-type PKR (pRcCMV/PKR) with pCMV-REP-*LacZ* decreased by 20-fold the amount of β-galactosidase activity (Fig. 9). Also PKR$^{-/-}$ MEFs showed increased β-galactosidase activity compared with wild-type MEFs transfected with pCMV-REP-*LacZ* (results not shown). Therefore, suppression of PKR in transfected cells significantly enhanced production of heterologous proteins from the viral-based vector.

DISCUSSION

Transfections and viral infections induce some of the same stress responses possibly because both treatments can result in the production of dsRNA. The synthesis of dsRNA from plasmid DNA can occur as a result of complementary RNA strands generated by transcription from cryptic or bidirectional promoters. Because dsRNA provides a signal to the cell that it has encountered a virus, the transfected cell responds as if it were infected. During viral infections, dsRNAs induce type I (α subtypes and β) interferons which enhance transcription of genes encoding PKR and the 2-5A-synthetases. Subsequently, dsRNA binds to and activates PKR and the 2-5A-synthetases leading to suppression of translation intiation and RNA degradation by RNase L, respectively (Fig. 1). However, the effectiveness of antiviral proteins is compromized by viral-encoded inhibitors (1). In contrast, during transfections PKR and RNase L are not inhibited and therefore large increases in gene

expression occur if these enzymes are absent (Fig. 2). While PKR has previously been indirectly implicated in the selective inhibition of transfected genes (3–7), the present study provides the first direct evidence for PKR involvement in control of exogenous gene expression. In addition, we show for the first time that the 2-5A/RNase L pathway also suppresses exogeneous gene expression. By taking advantage of cell lines from gene knockout mice, we have linked both PKR and RNase L to the control of exogenous gene expression. The high expression of plasmid-encoded genes observed in cells lacking PKR or RNase L was independent of the type of promoter and of the type of gene being expressed (Fig. 2). Enhanced levels of RNA from the transfected genes in the RNase L knockout cells was consistent with the stabilization of such transcripts, although other interpretations are possible (Fig. 3). Remarkably, the RNase $L^{-/-}$ cells expressed high levels of a hybrid CD4 mRNA fused to an AU-rich instability sequence from the GM-CSF 3′-UTR (18). Curiously, while fibroblasts from $PKR^{-/-}$/RNase $L^{-/-}$ embryos expressed more protein from exogenous genes than wild-type or RNase $L^{-/-}$ cells they produced less protein than did cells lacking only PKR. However, these findings may merely be an indication that the double knockout cells are less robust than the single knockout cells.

Suppression of vector-encoded gene expression by PKR and by the 2-5A system occurs without inhibiting endogenous gene expression (Fig. 5). The selective reduction in gene expression from plasmid vectors may be due to the synthesis of antisense RNA that anneals to the mRNA to form dsRNA. It has been proposed that such transcripts harboring dsRNA could lead to PKR activation and phosphorylation of eIF2α associated with the mRNA (25). Accordingly, binding of partially double-stranded mRNA to ribosomes was inhibited by localized activation of PKR. The 2-5A system can also discriminate between RNA molecules that contain double-stranded regions and single-stranded RNA (26). For example, partially dsRNAs, such as occur in some viral replicative intermediates, are selectively degraded by the 2-5A/RNase L pathway apparently due to localized activation of 2-5A-synthetases and of RNase L (26). Similarly, during picornavirus infections there was selective degradation by RNase L of viral RNA compared with cellular transcripts (27). Plasmid-derived transcripts appear also to be selectively degraded by RNase L (Fig. 3). Perhaps the double-stranded intermediates of the Semliki Forest virus vector provide a similar function by causing localized activation of 2-5A synthetase and PKR (Fig. 9).

Adenoviruses are resistant to the antiviral action of interferons due to suppression of signal transduction to the interferon stimulated genes by transcription factor E1A and from inhibition of PKR by VAI RNA (27,28). The inhibitory effect of VAI RNA on PKR has been exploited in a commercial vector for enhancing plasmid vectors by co-transfecting with pAdVAntage containing the VAI and II genes (Promega). However, the molecular mechanism for the enhancement in gene expression has been alternatively attributed to PKR inhibition or to stabilization of plasmid-derived transcripts (3,4,11,12). Results described in the present study indicated that accumulation of transcripts may be the predominant mechanism leading to enhanced gene expression (Figs 4, 6 and 7). Co-transfections with pAdVAntage produced elevated levels of plasmid-derived gene transcripts and protein even in cells lacking PKR (Fig. 4). Indeed, the VA RNA effect was several-fold greater in PKR knockout cells than in wild-type or RNase L knockout cells. The VA RNAs did not enhance translation in general, but rather caused a modest decrease (Fig. 5). There was an increase in levels of the plasmid-derived transcript in response to co-transfection with pAdVAntage in all of the cell types (Fig. 7). However, the β-galactosidase transcript accumulated to the highest levels in the $PKR^{-/-}$ cells co-transfected with pAdVAntage suggesting that stabilization of the mRNA was coupled to translation.

The suppressing effect of PKR on translation of plasmid vector transcripts was confirmed by expressing a dominant-negative PKR in wild-type or RNase $L^{-/-}$ cells (Fig. 8). In addition, co-transfections of the dominant-negative PKR with a self-replicating Semliki Forest virus-based vector enhanced expression of a transgene for β-galactosidase (Fig. 9). A much larger effect of human dominant-negative PKR was obtained in monkey (COS) cells as compared to hamster (BHK) cells, perhaps indicative of closer sequence homology between the human mutant and endogenous form of monkey PKR. Inhibition of PKR may be used to increase viral titers obtained from cell-based viral expression systems and could enhance expression of viral-encoded genes.

These results demonstrate that host enzymes that have evolved to control viral infections also limit the efficacy of gene expression systems. $PKR^{-/-}$ and RNase $L^{-/-}$ cells may prove useful for obtaining proteins from difficult to express genes and may even increase production of protein therapeutic agents compared with currently available cell culture systems. Therefore, suppression of PKR and RNase L is a promising approach for enhancing gene expression from viral- and non-viral-based vectors.

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