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Mechanism of interferon action: Autoregulation of RNA-dependent P1/eIF-2α protein kinase (PKR) expression in transfected mammalian cells

(translational control/protein synthesis factor eIF-2α/kinase phosphotransfer mutant/RNA-binding protein)

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ABSTRACT **The expression of a molecular cDNA clone (P1 KIN) of the human RNA-dependent P1/eIF-2α protein kinase (PKR) was examined in transfected monkey cells and in cell-free protein-synthesizing systems. Expression of the wild-type (wt) P1 KIN cDNA, which encodes an active protein kinase, was compared with that of the phosphotransfer catalytic domain II Lys-296 → Arg (K296R) mutant cDNA, which does not encode an active kinase. wt and K296R mutant P1 mRNAs prepared by transcription *in vitro* with T7 RNA polymerase programmed the cell-free synthesis of P1 ribosome-associated protein with comparable efficiency in the rabbit reticulocyte system. The K296R mutant P1 protein was also efficiently synthesized *in vivo* in transfected COS monkey cells. However, synthesis of the wt P1 protein was reduced about 30-fold in transfected COS cells as compared with the K296R mutant P1 protein. Cotransfection of wt P1 KIN cDNA with either K296R mutant P1 KIN cDNA or reovirus S4 cDNA greatly reduced the synthesis of K296R mutant P1 protein and reovirus σ3 protein, respectively. Although the wt and K296R mutant P1 KIN plasmid expression vectors replicated with comparable efficiencies in COS cells, the steady-state amount of P1 mRNA was about 3-fold less in COS cells transfected with the wt as compared with the K296R mutant P1 KIN cDNA. These results suggest that RNA-dependent P1 protein kinase expression is autoregulated *in vivo* in transfected mammalian cells primarily at the level of translation by a mechanism that is likely dependent upon catalytically active P1 kinase.**

Among the enzymes induced by interferon (IFN) is a protein-serine/threonine kinase, designated the P1/eIF-2α protein kinase, but also known as the P1 kinase, p68 kinase, DAI, dsI, and PKR (1, 2). The IFN-induced P1/eIF-2α kinase is dependent upon RNA for activation, a process which involves an RNA-dependent autophosphorylation of P1 ribosomal protein (3–6). When activated, the P1/eIF-2α kinase catalyzes the phosphorylation of the α subunit of protein synthesis initiation factor eIF-2 at Ser-51 (7, 8). Phosphorylation of eIF-2α causes an inhibition of protein synthesis at the initiation step of translation (9). Considerable evidence has accumulated consistent with the notion that the P1/eIF-2α kinase plays a central role in translational control and in the antiviral action of IFN (2, 10).

Molecular cDNA clones of the P1/eIF-2α protein kinase have been obtained from human amnion U cells (11) and human Daudi cells (12, 13). The deduced amino acid sequence of the cDNA predicts a 551-amino acid protein. The catalytic domains conserved among protein-serine/threonine kinases are present within the C-terminal half of the P1 protein. Transcripts prepared from cDNA clones of the kinase program the cell-free synthesis of a 67-kDa protein which possesses P1/eIF-2α protein kinase activity (12) and which is indistinguishable by immunoprecipitation and immunoblot gel analyses from authentic protein P1 synthesized in IFN-treated human cells (11). P1 protein synthesized from the cDNA clone P1 KIN, both in cell-free systems and in *Escherichia coli*, possesses RNA-binding activity which maps by deletion analysis to a domain within the N-terminal 98 residues of P1 (13–15). In contrast to the information gained from studying the expression of the P1 KIN cDNA in rabbit reticulocyte lysates and in bacteria, no information is yet available concerning the expression of P1 KIN cDNA in animal cells.

We report herein the expression of wild-type (wt) and mutant forms of the P1 KIN cDNA in transfected mammalian cells. The results suggest that the synthesis of wt P1 protein is autoregulated principally at the level of translation, whereas the synthesis of a catalytic domain II mutant P1 protein [Lys-296 → Arg (K296R)] deficient in kinase activity is not autoregulated.

EXPERIMENTAL PROCEDURES

Expression Plasmid Constructions. The molecular cloning of the RNA-dependent P1/eIF-2α protein kinase from human amnion U cells and the consensus sequence of the U cell full-length P1 KIN cDNA (GenBank accession no. M85294) have been previously reported (11). The general-purpose expression vector pJC119 (16) was used to express the P1 KIN cDNA within transfected COS cells by a scheme similar to that utilized to express full-length cDNA copies of reovirus S-class genes (17).

Plasmid vector pSVP1KIN(Wt) containing the complete coding region of the wt P1 protein was constructed by ligation of the 1.8-kilobase (kb) *Hin*dIII/*Pst* I P1 fragment from pBlue-P1KIN(Wt) (11) into the *Bam*HI site of the pJC119 expression vector. The ends of the *Hin*dIII/*Pst* I P1 fragment were blunted by using the Klenow fragment of *E. coli* DNA polymerase (for *Pst* I, incubation was overnight at 16°C without nucleotides, and for *Hin*dIII incubation was for 1 hr at 37°C with nucleotides), and the *Bam*HI-digested pJC119 vector was likewise blunted with the Klenow fragment prior to ligation.

Plasmid vector pSVP1KIN(K296R) containing the complete coding region of a K296R mutant P1 protein was constructed by replacing the *Bgl* II fragment of pSVP1KIN(Wt) with the *Bgl* II fragment isolated from plasmid pBlue-P1KIN(K296R). The correct orientation was confirmed by

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Abbreviations: IFN, interferon; eIF-2α, the α subunit of eukaryotic protein synthesis initiation factor 2; wt, wild-type; SV40, simian virus 40.

restriction analysis, and the presence of the mutation (K296R) was verified by direct sequence analysis.

Plasmid vectors pSVS1 and pSVS4 containing full-length cDNAs of the reovirus serotype 1 Lang strain wt *S1* gene (GenBank accession no. M14779) and *S4* gene (GenBank accession no. M13139) were previously described (17).

Oligonucleotide-Directed Mutagenesis. The point mutant of P1 protein kinase, K296R, was prepared by site-directed mutagenesis. Briefly, the *Bgl* II (721)/*Eco*RI (1099) fragment of the P1 KIN cDNA was subcloned in the pBluescript II SK (+) vector (Stratagene), and uracil-containing single-stranded template DNA was prepared by using *E. coli* CJ236 and the helper phage M13K07. The mutant oligonucleotide 5′-ATTTAACACGTCTAATAACG-3′ (minus-sense nucleotides 898–879), synthesized by using an Applied Biosystems model 380A automated DNA synthesizer, was annealed with the plus-sense template single-stranded DNA, extended with T7 DNA polymerase, and ligated with T4 DNA ligase. The resulting double-stranded DNA plasmid was used to transform *E. coli* MV1190; recombinants were directly sequenced by the dideoxynucleotide chain-termination method (18). The *Acc* I/*Bcl* I fragment containing the Lys-296 → Arg mutation was then introduced into the full-length pBlue-P1 KIN cDNA clone by restriction fragment exchange, resulting in the plasmid pBlue-P1KIN(K296R).

Cell Maintenance and Transfection. COS African green monkey kidney cells were cultured in monolayer as previously described (17, 19). The pSVP1KIN, pSVS1, and pSVS4 expression constructs were introduced into COS cells by the DEAE-dextran/chloroquine phosphate transfection method (17, 19, 20). All transfections were performed with DNA at 5 μg/ml.

Measurement of Protein Synthesis *in Vivo*. The synthesis of P1 protein and reovirus σ1 and σ3 proteins was measured at 48 hr after transfection by pulse-labeling with [^{35}S]methionine for 60 min, preparation of cell-free extracts by lysis with buffer containing Nonidet P-40, immunoprecipitation with a saturating amount of rabbit polyclonal antibody and Formalin-fixed *Staphylococcus aureus*, and analysis of the proteins by NaDodSO$_4$/polyacrylamide gel electrophoresis and autoradiography (11, 19). Protein synthesis was quantitated by scanning autoradiograms with an LKB Ultroscan XL laser densitometer. The methods have previously been described in detail (11, 19, 21).

Immunoblot Analysis. Western immunoblots were prepared by the method of Towbin *et al.* (22) as previously described (11).

Northern Gel-Blot Analysis. Northern gel-blot analysis of RNA transcripts was carried out as previously described (11).

Southern Gel-Blot Analysis. Southern gel-blot analysis (23) was carried out on DNA from transfected COS cells isolated by a modification of the Hirt procedure (24).

Preparation of P1 mRNA and Translation *in Vitro*. P1 mRNA transcripts were prepared as *in vitro* transcription products by using T7 RNA polymerase and the pBluescript vector construction pBlue-P1 KIN containing the full-length P1 KIN cDNA insert (11, 25). The structural integrity of the RNA transcripts was ascertained by formaldehyde/agarose gel electrophoresis followed by autoradiography.

The *in vitro* translation of P1 mRNA catalyzed by rabbit reticulocyte lysates (Promega) and the analysis of ^{35}S-labeled protein products were as previously described (25).

Materials. Unless otherwise specified, materials and reagents were as previously described (11, 14, 17).

RESULTS

Expression of the P1 KIN cDNA in Transfected Cells. To express the cDNA copy of the P1 protein kinase in mammalian cells, expression plasmids were constructed containing a 1.8-kb insert possessing the 551-amino acid open reading frame of the P1 KIN cDNA (11). The general-purpose eukaryotic expression vector pJC119 (16) was used, with P1 protein expression under the control of the simian virus 40 (SV40) late promoter (16).

P1 KIN expression constructs containing either the wt cDNA [pSVP1KIN(Wt)] or the catalytic domain residue 296 mutant cDNA [pSVP1KIN(K296R)] were introduced into COS monkey cells by the DEAE-dextran/chloroquine phosphate transfection method. Synthesis of P1 protein was then measured by pulse-labeling with [^{35}S]methionine. The K296R mutant P1 protein was efficiently synthesized in pSVP1KIN(K296R)-transfected cultures (Fig. 1*A*, lane 3). By contrast, the synthesis of wt P1 protein was far less efficient than that of the K296R mutant P1 protein (Fig. 1*A*, lanes 2 and 3).

Although the wt P1 protein was not efficiently synthesized in transfected COS cells, wt P1 protein was efficiently synthesized in rabbit reticulocyte lysates (Fig. 1*B*, lane 5). Furthermore, the wt P1 mRNA was translated *in vitro* with an efficiency comparable to that of the K296R mutant P1 mRNA (Fig. 1*B*, lane 5 as compared with lane 6). Quantitation of the amount of P1 protein synthesized *in vitro* at different mRNA concentrations, both in the presence and in the absence of the kinase antagonist 2-aminopurine, revealed less than a 2-fold difference in the translational efficiency between wt and K296R mutant P1 mRNAs (D.C.T., S. J. McCormack, J. P. Doohan, and C.E.S., unpublished observations).

The steady-state level of P1 protein was also examined in transfected cells by Western immunoblot analysis (Fig. 2). Rabbit immune serum, prepared against the recombinant P1

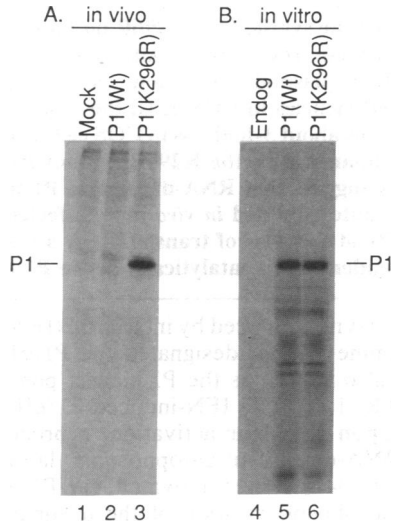

FIG. 1. Synthesis of recombinant wt and phosphotransfer K296R mutant P1 proteins *in vivo* and *in vitro*. (*A*) Synthesis of P1 protein in transfected COS cells. Monolayers of COS cells were transfected with the pSVP1KIN expression plasmids and 48 hr later cells were pulse-labeled with [^{35}S]methionine for 1 hr. Nonidet P-40 extracts were prepared, and P1 protein was immunoprecipitated and analyzed by NaDodSO$_4$/10% PAGE. Lane 1 (Mock), mock-transfected COS cells; lane 2 [P1(Wt)], wt pSVP1KIN(Wt)-transfected cells; and lane 3 [P1(K296R)], mutant pSVP1KIN(K296R)-transfected cells. (*B*) Synthesis of P1 protein in rabbit reticulocyte lysates. Capped P1 mRNAs, prepared from the pBlue-P1KIN and pBlue-P1KIN-(K296R) templates by transcription *in vitro*, were translated (4 μg/ml) by using the reticulocyte system, and the [^{35}S]methionine-labeled P1 protein product was immunoprecipitated and analyzed by NaDodSO$_4$/10% PAGE. Lane 4 (Endog), no exogenously added RNA; lane 5 [P1(Wt)], lysate programmed with wt P1 RNA; and lane 6 [P1(K296R)], lysate programmed with K296R mutant P1 RNA. The position of the 67-kDa P1 protein band is indicated.

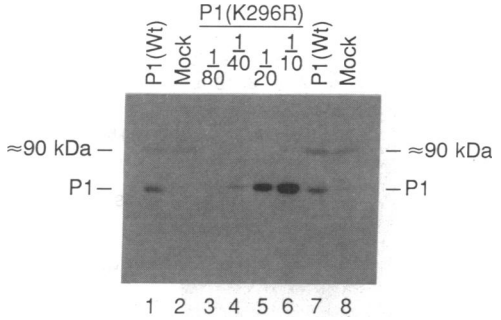

FIG. 2. Western immunoblot analysis of wt and K296R mutant P1 protein expressed in transfected COS cells. COS cells were transfected with the pSVP1KIN expression plasmids and unlabeled Nonidet P-40 extracts were prepared 48 hr later. Proteins were analyzed by $NaDodSO_4$/10% PAGE followed by Western immunoblotting (11, 22). Lanes 1 and 7 [P1(Wt)], wt pSVP1KIN(Wt)-transfected COS cells; lanes 2 and 8 (Mock), mock-transfected cells; lanes 3–6 [P1(K296R)], mutant pSVP1KIN(K296R)-transfected cells. Lanes 3, 4, 5, and 6 contained P1(K296R) extract diluted 1/80, 1/40, 1/20, and 1/10, respectively, relative to the amount loaded for the P1(Wt) lanes. The positions of the 67-kDa P1 protein band and a crossreacting ≈90-kDa protein band (11) are indicated.

protein synthesized in *E. coli* (11), readily recognized a 67-kDa protein present in unlabeled extracts prepared from pSVP1KIN(K296R)-transfected COS cells (Fig. 2, lanes 3–6). The steady-state amount of the K296R mutant P1 protein that accumulated in transfected cells was about 30-fold greater than the amount of wt P1 protein that accumulated in transfected COS cells, as revealed from the quantitation of dilution standards by laser densitometry (Fig. 2, lanes 1, 4, 5, and 7).

The immune serum raised against recombinant human P1 protein also detected an endogenous monkey P1 protein in extracts prepared from mock-transfected COS cells (Fig. 2, lanes 2 and 8). However, the Western signal of the endogenous monkey P1 was weak, even compared with that obtained for the wt human P1 protein in transfected cells. Finally, P1 immune serum also recognized in monkey COS cells an ≈90-kDa protein (Fig. 2) similar to that previously detected in human U cells. Preimmune serum did not recognize either protein P1 or the 90-kDa protein in U cells (11).

Effect of 2-Aminopurine on the Synthesis of P1 Protein in Transfected Cells. Among the inhibitors of P1/eIF-2α protein kinase function are 2-aminopurine, a purine analogue, and σ3, a reovirus RNA-binding protein (2).

Treatment of COS cells with 2-aminopurine beginning 24 hr after transfection did not enhance the rate of synthesis of either wt or K296R mutant P1 protein (Fig. 3, lanes 3–6). By contrast, as a positive control, treatment of pSVS1-transfected cells with 2-aminopurine increased the efficiency of reovirus σ1 expression encoded by s1 mRNA (Fig. 3, lanes 7 and 8); s1 mRNA is an activator RNA of the P1 kinase (26). However, treatment of pSVS4-transfected cells with 2-aminopurine under identical conditions did not alter the efficiency of reovirus σ3 expression (Fig. 3, lanes 9 and 10), as previously reported (19).

Although treatment of cells with 2-aminopurine beginning 24 hr after transfection did not affect either wt or K296R mutant P1 expression, when the 2-aminopurine treatment was begun only 3 hr after transfection and continued until the harvest at 48 hr, then the K296R mutant P1 expression was significantly reduced (Fig. 4, lane 4 versus lane 5). The expression of wt P1 was still not readily detected after a 45-hr treatment with 2-aminopurine (Fig. 4, lanes 2 and 3).

Expression of pSVP1KIN and pSVS4 Vectors in Cotransfected Cells. The reovirus σ3 protein prevents the RNA-dependent activation of the P1 kinase by complexing with

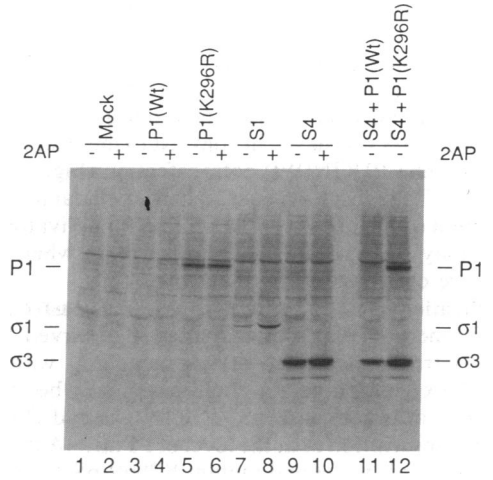

FIG. 3. Effect of 2-aminopurine and reovirus σ3 protein on the expression of wt and K296R mutant P1 proteins in transfected COS cells. COS cells were transfected with pSVP1KIN vectors and [^{35}S]methionine-labeled as described for Fig. 1*A*. Where indicated, cells were treated with 10 mM 2-aminopurine (2AP), beginning 24 hr after transfection. P1 proteins and reovirus σ1 and σ3 proteins were immunoprecipitated and analyzed by $NaDodSO_4$/10% PAGE. Lanes 1 and 2 (Mock), mock-transfected COS cells; lanes 3 and 4 [P1(Wt)], pSVP1KIN(Wt)-transfected cells; lanes 5 and 6 [P1(K296R)], mutant pSVP1KIN(K296R)-transfected cells; lanes 7 and 8 (S1), pSVS1-transfected cells; lanes 9 and 10 (S4), pSVS4-transfected cells; lane 11 [S4 + P1(Wt)], cells cotransfected with pSVS4 and wt pSVP1KIN(Wt); and lane 12 [S4 + P1(K296R)], cells cotransfected with pSVS4 and mutant pSVP1KIN(K296R). The positions of proteins P1, σ1, and σ3 are indicated.

candidate activator RNAs (2, 27). However, cotransfection of the reovirus *S4* gene (pSVS4) with the wt pSVP1KIN(Wt) vector surprisingly did not lead to an increase in the expression of the wt P1 protein (Fig. 3, lane 11). Rather, the synthesis of the reovirus σ3 protein encoded by pSVS4 was somewhat decreased in the cultures cotransfected with pSVP1KIN(Wt)

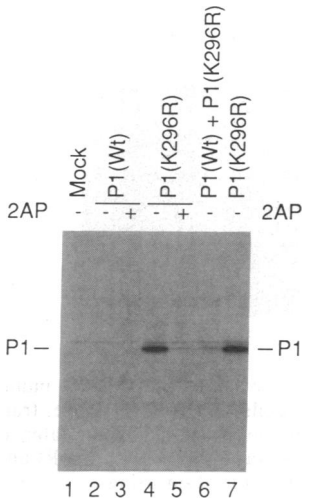

FIG. 4. Effect of 2-aminopurine and cotransfection on the expression of wt and K296R mutant P1 proteins in transfected COS cells. COS cells were transfected with the pSVP1KIN expression plasmids and the ^{35}S-labeled P1 protein product was analyzed as for Fig. 3. Where indicated, cells were treated with 10 mM 2-aminopurine (2AP), beginning after chloroquine treatment. Lane 1 (Mock), mock-transfected COS cells; lanes 2 and 3 [P1(Wt)], wt pSVP1KIN(Wt)-transfected cells; lanes 4, 5, and 7 [P1(K296R)], mutant pSVP1KIN(K296R)-transfected cells; and lane 6 [P1(Wt) + P1(K296R)], cells cotransfected with wt pSVP1KIN(Wt) and mutant pSVP1KIN(K296R). The position of protein P1 is indicated.

(Fig. 3, lane 9 versus lane 11). Similarly, the synthesis of the K296R mutant P1 protein was inhibited in cultures cotransfected with pSVP1KIN(Wt) and pSVP1KIN(K296R) (Fig. 4, lanes 2, 4, and 6). Cotransfection of the mutant pSVP1KIN(K296R) vector with the reovirus S4 gene did not affect reovirus σ3 synthesis, in contrast to the result obtained with the wt pSVP1KIN(Wt) cotransfection (Fig. 3, lanes 9, 11, and 12). These results suggest that the cellular localization of P1 protein or the affinity of P1 protein for activator RNAs, or both, may differ from that of σ3 protein when the two proteins are coexpressed in transfected cells.

Amplification of pSVP1KIN Plasmids in Transfected Cells. The difference in P1 protein synthesis observed between COS cells transfected with wt as compared with mutant K296R P1 expression vector could potentially be caused by a difference at the level of pSVP1KIN plasmid DNA replication or transcription or at the level of P1 mRNA translation. Amplification of the wt and mutant K296R pSVP1KIN plasmid vectors in transfected COS cells was not detectably different as measured by Southern blot hybridization of Hirt supernatant DNA fractions digested with *Eco*RI. The amount of the predicted 829-base-pair (bp) P1-specific fragment was comparable for DNA isolated from wt- and mutant-transfected cells when cultured in the absence of 2-aminopurine (Fig. 5, lanes 3 and 5). The fragment was not detected in mock-transfected cells (lane 2). Treatment of cultures with 2-aminopurine beginning 3 hr after transfection drastically reduced the amplification of both the wt and the mutant P1 vector plasmids (lanes 4 and 9) as compared with parallel cultures not treated with 2-aminopurine (lanes 3 and 5).

Northern Gel-Blot Analysis of P1 mRNA Expression in Transfected Cells. Northern gel-blot analysis revealed that the level of P1 mRNA was slightly greater in cells transfected with the P1 K296R mutant as compared with the P1 wt vector (Fig. 6, lanes 2 and 3). The P1 KIN cDNA probe hybridized to two P1-specific mRNAs in transfected cells, the predicted

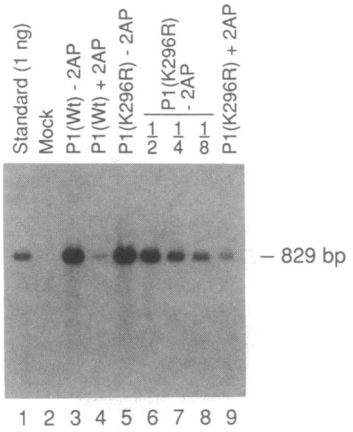

FIG. 5. Replication of wt and K296R mutant P1 plasmids in transfected COS cells. COS cells were transfected with the pSVP1KIN expression plasmids; 48 hr later Hirt extracts were prepared, and DNA was digested with *Eco*RI and then analyzed by Southern gel-blot hybridization using as the probe the ^{32}P-labeled 0.8-kb *Eco*RI fragment of the P1 KIN cDNA at 1×10^6 cpm/ml. Where indicated, cells were treated with 10 mM 2-aminopurine (2AP) beginning after chloroquine treatment. Lane 1 (Standard), 1 ng of pSVP1KIN(Wt) plasmid digested with *Eco*RI; lane 2 (Mock), DNA from mock-transfected COS cells; lanes 3 and 4 [P1(Wt) −/+ 2AP], DNA from wt pSVP1KIN(Wt)-transfected cells cultured in the absence (−) or presence (+) of 2AP; and lanes 5–9 [P1(K296R) −/+ 2AP], DNA from mutant pSVP1KIN(K296R)-transfected cells cultured in the absence (−) or presence (+) of 2AP. Lanes 6, 7, and 8 contained 1/2, 1/4, and 1/8, respectively, of the amount of DNA sample analyzed in the other lanes. The position of the 829-bp *Eco*RI fragment standard is indicated.

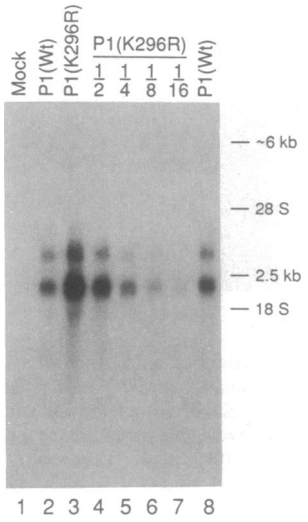

FIG. 6. Steady-state level of plasmid-derived P1 mRNA in transfected COS cells. COS cells were transfected with the pSVP1KIN expression plasmids; 48 hr later total cellular RNA was isolated (28) and analyzed by Northern gel-blot hybridization using the 1.8-kb *Hin*dIII/*Pst* I fragment of the P1 KIN cDNA (11) as the probe. Lane 1 (Mock), RNA from mock-transfected COS cells; lanes 2 and 8 [P1(Wt)], RNA from wt pSVP1KIN(Wt)-transfected cells; and lanes 3–7 [P1(K296R)], RNA from mutant pSVP1KIN(K296R)-transfected cells. Lanes 4, 5, 6, and 7 contained 1/2, 1/4, 1/8, and 1/16, respectively, of the standard amount (5 μg) of total RNA. The position of the 18S and 28S ribosomal RNAs and the 2.5-kb and ≈6-kb P1 transcripts observed in human amnion U cells are indicated.

2.3-kb species which was the major P1-specific mRNA, and a minor P1 mRNA of about 2.8 kb. The minor mRNA is likely a splice-site variant of the SV40 leader specified by the pJC119 vector (29). Quantitation of dilution standards by laser densitometry revealed that the steady-state amount of mutant P1 mRNA was 3-fold greater than that of wt P1 mRNA in transfected cells (Fig. 6, lanes 2, 4, and 5).

DISCUSSION

In this paper we provide information regarding the successful expression, and the regulation, of human P1/eIF-2α protein kinase cDNA clones in mammalian cells. Two important points emerge from the studies. First, using transient transfection assays, we demonstrated the synthesis of both the wt and a phosphotransfer mutant (K296R) P1 protein in transfected COS monkey cells. Second, the K296R mutant P1 protein was synthesized at a greatly elevated level in COS cells relative to the wt P1 protein, whereas in cell-free protein-synthesizing systems the wt and K296R mutant P1 mRNAs were translated with comparable efficiencies.

Approximately 30-fold more K296R mutant P1 protein was synthesized than wt P1 protein in COS cells transfected with pSVP1KIN cDNA expression plasmids. Systematic analyses revealed that the DNA template copy number was comparable for the K296R mutant and wt P1 plasmids and that the steady-state amount of the K296R mutant P1 mRNA was only about 3-fold greater than that of wt P1 mRNA. However, the amount of mutant K296R P1 protein was at least 30-fold greater than that of wt P1 protein. This difference in amount of P1 protein likely reflects a difference in rate of P1 protein synthesis, rather than degradation, because both pulse-labeling with [^{35}S]methionine and Western immunoblot analysis revealed a comparably enhanced level of P1 protein in mutant- as compared with wt-transfected cells.

Whereas a part of the increased amount of mutant P1 protein may be attributed to the 3-fold higher transcript level,

most of the 30-fold difference between wt and mutant P1 protein levels is due to an increased translational efficiency of the mutant P1 mRNA. No difference in replication of K296R mutant and wt plasmid DNA vectors was observed. The differential expression of the K296R mutant P1 protein over that of the wt P1 protein is not limited to plasmid-derived P1 mRNAs expressed from the SV40 late promoter-enhancer in COS cells containing the pJC119 vector as described herein. Similar results have been obtained with the baculovirus vector pEVmXIV and Sf21 insect cells (30); baculovirus recombinants are readily generated that efficiently express the K296R mutant P1 protein but not the wt P1 protein (D.C.T. and C.E.S., unpublished observations).

These observations regarding the differential efficiency of expression of wt and K296R mutant P1 kinase may be related to the effects of IFN on the expression of plasmid-derived mRNAs in COS cells. IFN treatment inhibits the expression of vesicular stomatitis virus *G* gene and reovirus *S3* gene in pSVG- and pSVS3-transfected COS cells at the level of translation (29, 31). Furthermore, disruption of the RNA-dependent P1 kinase pathway—for example, by expression of kinase antagonists such as adenovirus VA RNA or eIF-2α subunit mutants (S51A) or homologues (K3L)—increases the translational efficiency of plasmid-derived mRNAs in COS cells (32, 33).

The reason why the K296R point mutant, which lacks P1 kinase activity, would give about a 3-fold higher P1 transcript level in transfected cells than the wt P1 kinase is unclear, but it may indicate a role for the P1 kinase in the modification of a transcriptional component utilized by the SV40 late promoter-enhancer. Consistent with this possibility, the expression of adenovirus VAI RNA, an antagonist of the P1 kinase (2), in monkey CV1p cells causes a 3- to 4-fold increase in the synthesis of SV40 tumor (T) antigen mRNA (34).

Autoregulation of gene expression is an important mechanism in mammalian cells. Although autoregulation was first clearly demonstrated at the transcriptional level, as exemplified by SV40 T antigen expression (35), the mRNA translation level is also a step for control of expression by autoregulation. The synthesis of the iron binding and storage protein ferritin, for example, is primarily regulated at the translational level (36). The apparent autoregulation of P1 expression is dependent upon P1 kinase catalytic activity and not simply P1 RNA-binding activity. Both wt P1 and mutant K296R P1 possess RNA-binding activity but only wt P1, which was autoregulated, is an active kinase (D.C.T., S. J. McCormack, J. P. Doohan, and C.E.S., unpublished observations, and refs. 13–15).

It is well established that the RNA-dependent P1 kinase (PKR) can selectively bind to, and be regulated by, certain structured single-stranded RNAs (2). Conceivably the RNA-dependent P1 protein kinase binds to and is activated by its own mRNA, thereby autoregulating P1 protein synthesis at the level of translation. This autoregulation of a key enzyme of the IFN-induced antiviral response (2, 10) could be especially important during the period of host recovery from virus infection, when perhaps higher levels of P1 protein are no longer beneficial to the cell.

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