Characterization of Transgenic Mice with Targeted Disruption of the Catalytic Domain of the Double-stranded RNA-dependent Protein Kinase, PKR*

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Ninan Abraham, a,b,c David F. Stojdl, a,b Peter I. Duncan, d Nathalie Méthot, e Tetsu Ishii, f Manon Dubé, c Barbara C. Vanderhyden, c Harold L. Atkins, a,g Douglas A. Gray, a,b Michael W. McBurney, a,b Antonis E. Koromilas, f Earl G. Brown, b Nahum Sonenberg, a,b and John C. Bell a,b,c,h

From the aOttawa Regional Cancer Research Laboratories, Ottawa, Ontario K1H 8L6, the bDepartment of Biochemistry, University of Ottawa, Ottawa, Ontario K1H 8M5, Canada, the cDepartment of Molecular Biology, University of Geneva, 30 Quai Ernest-Ansermet, CH-1211 Geneva, Switzerland, the dDivision of Hematology, Ottawa General Hospital, Ottawa, Ontario K1H 8L6, the eDepartment of Oncology and Medicine, Lady Davis Institute for Medical Research, Montreal, Quebec H3T 1E2, the fDepartment of Microbiology and Immunology, University of Ottawa, Ottawa, Ontario K1H 8M5, and the gDepartment of Biochemistry, McGill University, Montreal, Quebec H3G 1Y6, Canada

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The interferon-inducible, double-stranded RNA-dependent protein kinase PKR has been implicated in anti-viral, anti-tumor, and apoptotic responses. Others have attempted to examine the requirement of PKR in these roles by targeted disruption at the amino terminal-encoding region of the Pkr gene. By using a strategy that aims at disruption of the catalytic domain of PKR, we have generated mice that are genetically ablating for functional PKR. Similar to the other mouse model of Pkr disruption, we have observed no consequences of loss of PKR on tumor suppression. Anti-viral response to influenza and vaccinia also appeared to be normal in mice and in cells lacking PKR. Cytokine signaling in the type I interferon pathway is normal but may be compromised in the erythropoietin pathway in erythroid bone marrow precursors. Contrary to the amino-terminal targeted Pkr mouse, tumor necrosis factor α-induced apoptosis and the anti-viral apoptosis response to influenza is not impaired in catalytic domain-targeted Pkr-null cells. The observation of intact eukaryotic initiation factor-2α phosphorylation in these Pkr-null cells provides proof of rescue by another eukaryotic initiation factor-2α kinase(s).

The interferon (IFN1)1-inducible protein kinase PKR is a well characterized effector of anti-viral responses in mammals (1, 2). Activation by double-stranded RNA (dsRNA) or stem loop RNA structures results in autophosphorylation and subsequent phosphorylation of the α subunit of eukaryotic initiation factor 2 (eIF-2α) (3–8). This phosphorylation causes sequestration of the guanine nucleotide exchange factor eIF-2B which prevents the exchange of GDP for GTP on eIF-2 and thereby inhibits translation initiation (9, 10). The decline in protein synthesis rates is deleterious to virus replication, and various viral mechanisms exist to circumvent inhibition of translation by PKR (11–18). Although there has been a considerable focus on its anti-viral role, PKR has been implicated in regulating other cellular functions such as differentiation (19), transcription (20–24), signal transduction (25), apoptotic response (26–28), and cell growth (29–32). For example, in yeast expression of PKR results in a slow growth phenotype (29), whereas expression of catalytically inactive PKR results in malignant transformation of NIH 3T3 cells (30–32). Interestingly, the p58ik6 inhibitor of PKR (33), the TAR RNA-binding protein inhibitor of PKR (34), and a non-phosphorylatable variant of eIF-2α (35) also transform NIH 3T3 cells underscoring the importance of translation initiation control and the regulation of cell growth.

PKR overexpression in HeLa cells induces apoptosis (28) by mechanisms that are inhibitable by Bcl-2 (27). Antisense ablation of PKR conferred resistance to tumor necrosis factor α (TNF-α)-induced apoptosis in U937 cells (36) indicating the requirement for PKR in the apoptotic response to TNF-α in these cells. It is thought that dsRNA is a trigger for apoptosis in vaccinia virus-infected cells (37), and influenza-mediated apoptosis is suppressed in cells expressing inactivated PKR (26).

Much of the work elucidating the role of PKR in growth control and apoptosis utilized mutated PKR in tissue culture settings. Previous work on targeted disruption of Pkr by homologous recombination in mice focused on interruption of two exons including one that encodes the initiating methionine (25). Surprisingly, analysis of this PKR-defective mouse model revealed no evidence of tumors and normal anti-viral responses in untreated animals. The mice were defective in IRF-1 and NF-κB signaling and showed diminished stress-induced apoptotic responses (20, 25, 38). To examine whether PKR is essential in anti-viral response, anti-proliferative functions of cellular growth control, and in apoptotic response to various stimuli, we generated mice devoid of PKR function by targeted disruption of the PKR catalytic domain using homologous recombination that interrupts exon 12. Mice homozygous for Pkr...
disruption (Pkr<sup>-/-</sup>) develop normally and are fertile with average sized litters. IFN-α and -β induction of transcription is intact, and the mice show normal hematopoiesis. Pkr<sup>-/-</sup> mice show responses to vaccinia and influenza infection comparable to control animals or cells. Apoptotic response to influenza infection or TNF-α was not impaired. Our data indicate that catalytic disruption of Pkr is not sufficient to ablate eIF-2α phosphorylation and that unappreciated members of the eIF-2α kinase family must compensate for loss of PKR function.

**MATERIALS AND METHODS**

**Construction of the Pkr Gene-targeting Vector—**A 28.8-kb region of isoegenic DNA encoding murine Pkr was isolated and mapped. A 2.9-kb SacI fragment that encodes exons 10 and 11 (39) as determined by sequence analysis and a 1.9-kb XhoI-BamHI fragment that bears intron XII sequence just distal to exon 12 were cloned into the MCSI neo poly(A) vector (Stratagene). The herpes simplex virus thymidine kinase expression cassette was introduced into the 3' end of the vector at the NotI site to produce the PKR catalytic domain replacement vector designated pTV65TK. Insertion of the neomycin (neo) cassette results in replacement of a 3.7-kb BamHI to XhoI fragment of the Pkr gene including complete removal of the 180-nucleotide exon 12.

**Generation of Pkr-deficient Mice—**The targeting vector was introduced into the J1 embryonic stem (ES) cell line (strain 129/terSv) (40) by electroporation, and cells were selected with neomycin (200 μg/ml) and 1-[(2-deoxy-2-fluoro-1-β-arabinofuranosyl)-5-iodouracil (0.2 μM); kindly provided by Dr. Michael A. Rudnicki) as described previously (41). Targeted disruption of the Pkr gene was determined by EcoRI or PstI digestion followed by Southern hybridization with probe A or probe B, respectively (see Fig. 1). To verify that only one copy of each construct was integrated in each targeted ES clone, XhoI-digested DNA was probed with the Neo probe. 3 out of 750 ES clones screened were true homologous recombinants. ES cells that were homozygous for the targeted Pkr allele were obtained by selection of the heterozygous targeted clone 7A8.3 in high neomycin selection (2 mg/ml as described previously (42)). Of 272 resistant colonies picked, one clone (7A8.3.7) was intact, and the mice show normal hematopoiesis.

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**Vaccinia Virus Growth in Cell Lines—**CV-1, L-929, wild-type, or Pkr-null cells were plated in duplicate at 2 × 10<sup>5</sup> cells per well in 6-well tissue culture plates. The next day, cells were infected with trypsin-treated wild-type vaccinia virus (WE strain) at an m.o.i. of 10 for 1 h at 37 °C. Following incubation, monolayers were rinsed and the inoculum was removed. Cells were frozen at −80 °C. Cells were scraped off in 0.3 ml of serum-free media and freeze-thawed twice before trypsinization and serial dilution. Plaque assays were performed, and after 2 days of incubation, plates were fixed, stained with crystal violet, and plaques counted to determine the number of pfu/ml of cell lysate as a function of time after infection.

**Influenza Infection of Pkr-null Mice—**Five-week-old Pkr<sup>-/-</sup> heterozygous control, Clk1-null, and Pkr-null mice were anesthetized with halothane and infected by intra-nasal inoculation with 50 μl of serial dilutions of the W29 strain of influenza in phosphate-buffered saline. Each group contained 8–12 animals that were monitored daily over a 10-day period. The W29 strain is pneumovirus for mice due to mutations selected on mouse adaptation of the prototype human influ- enza A strain, A/FM/1/47 (43). Influenza virus stocks were prepared in chicken embryo allantoic cavity, and infectivity titers were assessed by plaque assay on Madin-Darby canine kidney cells (43). The LD<sub>50</sub> value of the virus in the control and Pkr-null animals was assessed by Karber-Spearman analysis. Influenza virus growth in mouse lung was determined from pools of three mice from each group.

**Characterization of Transgenic Mice**

**Primary Mouse Embryo Fibroblasts (MEFs)—**Primary MEF cultures were established from E13 embryos as described previ- ously (40). Cells were genotyped as above. Immunoblot and Immune Complex Kinase Analysis of Pkr-null Cells—Wild-type and homogygous-null ES and MEF cells were lysed in Lysis buffer (10 mM Tris (pH 7.5), 150 mM sodium chloride, 5 mM EDTA, 1% Triton X-100), lysates denatured, resolved by SDS-PAGE, and blotted onto nitrocellulose membranes. PKR polypeptides were analyzed by immunoblotting with antisera (anti-Tik100A) raised against the immune complex with protein A-Sepharose beads. The complexes were resuspended in reaction mixtures of kinase buffer with 5 μCi of [γ<sup>32</sup>P]ATP and 10 ng/ml reovirus dsRNA and incubated at 25 °C for 30 min. Autophosphorylation activity of PKR was visualized by SDS-PAGE and phosphorimaging.
of Pkr-null and wild-type cells were washed and lysed in RIPA buffer with protease and phosphatase inhibitors. After clearing the lysates, 20 μg of protein were resolved by SDS-PAGE and transferred to nitrocellulose. Phosphorylation of eIF-2α was determined by immunoblot with a polyclonal anti-eIF-2α phosphoserine 51 antibody, whereas protein levels of eIF-2α were determined with a monoclonal antibody to eIF-2α. Normalization of protein levels was with anti-actin antibody.

RESULTS

Generation of Mice with Mutated Pkr Gene—We designed a targeting vector such that it would replace exon 12 of PKR (following the nomenclature of Kuhnen et al. (39)) with an HSV TK gene promoter-driven neomycin resistance cassette in an orientation opposite to the Pkr promoter (Fig. 1A). Exon 12 encodes subdomains V and VI of the catalytic domain and is essential for enzyme activity (30). Transfected ES cells were grown in selection media, picked, and screened for true homologous recombinants (Fig. 1B and data not shown) by Southern blot analysis. Targeted cell lines showed the predicted sized fragment for single site integration as detected by the internal blot analysis. Targeted cell lines showed the predicted sized fragment for single site integration as detected by the internal blot analysis. Homozygous Pkr-null cell line 7A8.3.7 was derived by high dose G418 selection. +/+; wild-type; +/−, heterozygous for Pkr-null allele; 0/0, homozygous for Pkr-null allele.

Characterization of Targeted Disruption of the PKR Catalytic Domain—An ES cell line (7A8.3.7) that was homozygous Pkr<sup>−/−</sup> was selected from the heterozygous mutant cell line, 7A8.3, as described previously (42), using higher dose G418 selection (Fig. 1B). These cell lines and primary mouse embryo fibroblasts (MEFs) derived from homozygous null animals were assessed for PKR activity. Northern blot analysis of poly(A)+-selected RNA from wild-type ES cells showed the normal pattern of murine PKR transcripts of 6, 4, and 2.5 kb that are induced 2.5–3-fold in IFN-treated cells (Fig. 2 (44)). The 4- and 6-kb and larger minor mPKR transcripts appear to be incompletely spliced since they hybridize to an intron-spanning riboprobe (data not shown). Heterozygous Pkr<sup>+/−</sup> cells showed similar expression of transcripts. Homozygous Pkr<sup>−/−</sup>...
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FIG. 2. Characterization of the Phr-targeted allele. Poly(A)\(^+\) Northern blot analysis of targeted Phr allele. mRNA was isolated from ES cells untreated or treated with IFN-\(\alpha\) and -\(\beta\) as indicated. \(+/+\), wild-type J1 cells; \(+/0\), heterozygous 7A8.3 cells; 0/0, homozygous TAR-3.T cells. Left panel, hybridization with murine PKR (mPKR) cDNA probe spanning exons 4–16, from nucleotides 290 to 1621; bottom panel, hybridization with glyceraldehyde phosphate dehydrogenase (GAPDH) cDNA; right panel, hybridization with neomycin (Neo) cDNA. Arrows indicate the 4-kb novel transcript and the 0.9-kb HSTK-neo derived transcript from the targeted allele.

A

B

FIG. 3. Immunoblot analysis of Phr-null ES and MEF cells demonstrate ablation of PKR protein. A. Western analysis. Total cell lysates from wild-type (+/+ or) or homozygous Phr-null (0/0) cells were extracted, separated by SDS-PAGE, immunoblotted with anti-mPKR antibody (affinity purified anti-TikMAP2 antiserum), directed against residues 101–114, and visualized with ECL. Lane 1 loaded with 25 \(\mu\)g of total protein; lanes 2–5 loaded with 100 \(\mu\)g of total protein. B, dsRNA affinity binding enrichment analysis. Total protein from the indicated ES cell lines were incubated with pIC-agarose; washed and bound protein were analyzed by immunoblot analysis. Anti-mPKR antiserum (anti-Tik100A) raised against purified His-mPKR residues 1–98 show mature PKR polypeptide in wild-type (+/+ or) and heterozygous (+/0) cells but no immunoreactive PKR polypeptides in homozygous (0/0) cells. Lane 1, 1.5 \(\mu\)g of total protein; lane 2, 15 \(\mu\)g of total protein; lane 3, 50 \(\mu\)g of total protein incubated with dsRNA. Molecular mass markers (kDa) are indicated on the left, and the 65-kDa murine PKR polypeptide (mPKR) is indicated on the right of each panel.
Furthermore, IFN induction of the 4-kb transcript in hematopoietic development in IRF-1 and NF-κB and cytokine signaling (20–24). PKR is implicated in regulating evidence points to a role for PKR in transcription control and factor treatment (data not shown). Erythroid CFU growth from Myeloid bone marrow precursors showed comparable response hematopoietic precursor population to cytokine stimulation. Later for color and counted to determine the response of each colony forming assays in the presence of the indicated cytokines. Six animals of each genotype were analyzed with triplicate platings of each cytokine treatment. CFU were scored 10 days after plating with cytokine. Error bars represent standard error of the mean. G-CSF, granulocyte-colony-stimulating factor.

PKRnull animals, however, demonstrated a reproducible diminished response to erythropoietin (Epo). IL-3 and KL co-treatment appear to be able to overcome this PKR-dependent Epo block in erythroid precursors from Pkrnull animals (Fig. 5). These precursors show a higher fold stimulation of CFU in response to IL-3 and KL than in wild-type bone marrow. This is consistent with a study implicating PKR in IL-3 stimulation of protein synthesis in an IL-3-dependent cell line (46). This block appears to have little physiological impact since hematocrit volumes from Pkr-null animals were unaffected (data not shown).

Pkrnull Animals Retain Anti-viral Responsiveness—We wished to determine if loss of PKR would affect the ability of null mice to counter viral challenge. Vaccinia maintains resistance to IFN despite producing large amounts of dsRNA in late infection (47). This is thought to be due to the production of K3L and E3L, two virally encoded PKR inhibitors (48, 49). The growth curve of vaccinia (WR strain) in wild-type and Pkr-null primary MEF cells in culture was examined (Fig. 6). The production of infectious vaccinia virus particles in control CV-1 and L-929 cells closely matched the kinetics of growth of this virus strain in HeLa cells (50). The virus replicated equally well in wild-type primary MEFs and, surprisingly, showed no significant difference in growth curve in PKR-deficient MEFs (Fig. 6 and inset). No enhancement of vaccinia yield was evident in the absence of PKR, in contrast to the repressive effect of overexpression of PKR (50).

In order to determine if replication of an RNA virus might be more affected by the loss of PKR, we examined the LD50 of the W29 mouse-adapted strain of influenza virus by intranasal infection of control and Pkrnull animals. As shown in Table II, heterozygous and Pkr-null animals showed a LD50 of 104.3 and 104.0 infectious particles, respectively. This 2-fold difference in apparent susceptibility of Pkrnull animals does not seem significant when compared, for example, to the consequences of loss of STAT1 function upon anti-viral response (51, 52). STAT1-deficient mice completely succumbed to doses of vesicular stomatitis virus challenge that were 104 to 105 lower than doses that were sublethal to wild-type animals. Furthermore, these animals had elevated susceptibility to a bacterial pathogen, Listeria, and opportunistic infections by murine hepatitis virus. We also measured the level of influenza growth in mouse lung 2 days post-infection for pools of three mice from each group. Pkr-null mice had 3.8 × 107 versus 9.0 × 107 pfu/ml for control mice. To date, we have found no sign of impaired anti-viral response or opportunistic infections in these animals. Our

![Image](50x489 to 296x729)

**FIG. 4. Immune complex kinase assay demonstrates loss of PKR catalytic activity in Pkrnull cells.** PKR protein was recovered by incubating lysates from the indicated wild-type and homozygous Pkr-null ES and MEF cells with anti-mPKR antiserum (anti-Tik100A).Immune complexes were incubated in reaction buffer with reovirus dsRNA and [γ-32P]ATP and following autophosphorylation of the kinase, resolved by SDS-PAGE and visualized. Lanes 1, 2, 4, 5, 7, and 8, 200 μg of total protein; lanes 3 and 6, 10 μg of total protein. Lane 1, protein A-Sepharose beads alone; lane 2, preimmune antiserum and protein A-Sepharose beads; lanes 3–8, anti-Tik100A and protein A-Sepharose beads. Molecular mass markers (kDa) are indicated on the left and the 65-kDa murine PKR polypeptide (mPKR) indicated on the right.

![Image](50x335)

**FIG. 5. Hematopoietic development in Pkrnull animals appears normal.** Erythroid bone marrow precursors were assessed for cytotoxic responsiveness by clonogenic assay in the presence of the indicated cytokines. Six animals of each genotype were analyzed with triplicate platings of each cytokine treatment. CFU were scored 10 days after plating with cytokine. Error bars represent standard error of the mean. G-CSF, granulocyte-colony-stimulating factor.
data demonstrate that PKR is not essential for IFN type I responses or for countering vaccinia or influenza infections and may be redundant in function.

**Virus-induced Apoptosis in PKR<sup>0/0</sup> Cells Is Unimpaired**—The role of PKR in regulating apoptotic responses has been shown by various groups ((26–28, 36, 53) see Introduction), and we wished to determine if PKR may curtail viral infections by inducing apoptosis.

To this end, PKR<sup>0/0</sup> MEFs were challenged with a strain of influenza A/HK/1/68 (H3N2) (54) at various m.o.i. Cells undergoing apoptosis were monitored using Annexin V-FITC (Boehringer Mannheim) staining, an early apoptosis marker (55). FACS analysis of MEFs infected with virus (Fig. 7) show a dose-dependent increase in cells undergoing apoptosis as determined by the elevated number of FITC-positive cells. The percentage of these cells also showing propidium iodide uptake, indicating incidental necrotic death, was small (typically 10–20% of total cells) (data not shown). PKR<sup>0/0</sup> cells were indistinguishable from wild-type cells in triggering apoptosis upon influenza infection. This contrasts with the effect of dominant-negative mutants of PKR that reduced influenza-mediated cell death (26). IFN-α/β pretreatment partially abrogated influenza-induced apoptosis in both normal and PKR-null cells up to an m.o.i. of 10 (Fig. 7). At higher m.o.i., IFN pretreatment was unable to rescue cells from virally induced apoptotic pathways. These data indicate that PKR is not essential for influenza to trigger apoptosis pathways in cells and that type I IFNs can mount anti-apoptotic responses at low m.o.i. independent of PKR.

**Stress-induced Apoptotic Response in PKR<sup>0/0</sup> Cells Is Normal**—Apoptosis may be induced by a number of signals including cellular stress. PKR itself has been implicated in responses to various forms of stress (56, 57). Der et al. (38) describe the abrogation of stress-induced apoptosis in the PKR-null cells they generated (25). The cells had impaired apoptotic responses to TNF-α, pIC, and lipopolysaccharide (LPS). We addressed the role of PKR in these pathways by determining whether our PKR-null cells were likewise impaired. We first assessed the dose response of our wild-type and PKR<sup>0/0</sup> MEFs to actinomycin D (Fig. 8A) which is required to prime cells to respond to these stimuli (38, 58, 59) (Fig. 8B). By having found no significant differences, we then optimized TNF-α, LPS, and pIC treatments of wild-type MEFs to trigger cell death using trypan blue exclusion (Fig. 8B) as described by Der et al. (38) and verified by TUNEL assay (data not shown). We found that at the cytostatic dose of actinomycin D (50 ng/ml) described previously (38), none of these treatments elicited much response from the MEF cells, even at the maximal doses of TNF-α, LPS, or pIC (Fig. 8B). It required higher doses of co-treatment with actinomycin D (500 ng/ml) before any effect was seen on cell viability, and this was restricted to TNF-α treatment only. Finally, we determined the dose response of wild-type and PKR-null MEFs to TNF-α-induced apoptosis and found that PKR<sup>0/0</sup> cells with disruption of the catalytic domain had no impairment of cell death response (Fig. 8C) as described in the other PKR-null mouse model (38). This was apparent throughout the dose range of TNF-α (0.1–20 ng/ml) used (Fig. 8C and inset).

**Intact eIF-2α Phosphorylation in PKR<sup>0/0</sup> Cells May Indicate the Presence of a Pkr Homolog**—The best known mammalian Pkr homolog, the heme-regulated inhibitor, is restricted to cells of erythroid lineage (60–62). The only other known homolog of Pkr is the yeast GCN2 kinase that is activated by uncharged tRNA and regulates amino acid biosynthesis by control of translation of the GCN4 transcript (63). Despite apparent specialization in function with unique regulatory domains, both mammalian eIF-2α kinases, heme-regulated inhibitor, and PKR can rescue GCN2-defective yeast (64). Since our knock-out strategy effectively renders the PKR catalytic domain inactive,
any eIF-2α phosphorylation in homozygous null cells must result from a previously unappreciated kinase activity. We used an antibody specific to phosphorylated eIF-2α (Fig. 9) (65) and determined the phosphorylation status of eIF-2α in both wild-type and Pkr-null animals. As shown in Fig. 9, both cell populations contain similar levels of eIF-2α, and there is little or no difference in eIF-2α phosphorylation status between wild-type and Pkr-null cells.

**FIG. 7.** Apoptotic response to influenza infection is unimpaired in Pkr-null cells. Untreated or IFN-pretreated MEFs were mock-infected or infected with influenza A/HK/1/68 virus at increasing m.o.i. After 24 h, cells were isolated, stained with Annexin V-FITC, fixed, and analyzed by FACS for cells undergoing apoptosis. Histograms showing distribution of fluorescence of cells of each genotype and treatment are shown. Left panels, wild-type (+/+ ) MEFs; right panels, Pkr-null (0/0) MEFs. Black trace, cells without IFN pretreatment; gray trace, IFN-α- and -β-pretreated cells.
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DISCUSSION

Targeted disruption of a Pkr, catalytic domain exon was performed to explore fully the consequences of loss of Pkr function at the organismal level. Our targeting strategy inactivates the catalytic domain by replacing an exon which encodes 60 amino acid residues including 6 amino acids that are known to be required for catalytic function (30). If any splicing variants exist that would terminate the reading frame, no mature or truncated polypeptide was observed in Pkr<sup>-/-</sup> cells, and PKR catalytic activity was undetectable. Mice homozygous for this Pkr disruption were bred and appeared to have no obvious defects in gross anatomy and were fertile with average sized litters. No evidence of susceptibility to opportunistic infections or signs of increased tumorigenesis have been observed.

The maintenance of proper control of translation is critical to cellular growth control. Indeed, it has been reported that translation restrictive elements are found in a disproportionate number of the untranslated regions of transcripts of proto-oncogenes, growth factors, hormone receptors, and transcription factors (69). The absence of any tumorigenic phenotype in either our Pkr-null mice or that of Yang et al. (25) was puzzling given the evidence of the consequences of translation deregulation. For instance, when the restriction of translation at the cap-binding step is circumvented by overexpression of the Cap-binding protein, eIF-4E, cellular transformation occurs (70). PKR restricts growth when expressed in yeast and causes morphological transformation when inactive mutated versions are expressed in mammalian cells. Furthermore, expression of non-phosphorylatable eIF-2α mutated at the serine 51 position (71, 72) and TAR-binding protein (33, 34).

Thus, the surprising absence of tumors in Pkr<sup>-/-</sup> mice suggests a redundancy of PKR function that has been previously unappreciated.

The anti-viral function of PKR has been studied with analyses of virus mutants lacking anti-PKR inhibitors as well as...
overexpression of Pkr. Strategies utilized by viruses to over-
come Pkr activation include inactivating RNAs, pseudosub-
strates, endogenous inhibitor mobilization, Pkr expression
down-regulation, and dsRNA sequestration. Certain viruses
have also developed means to prevent cell death responses
to infection such as cowpox crmA (73) and baculovirus IAP (74). A
couple of studies now implicate Pkr in controlling apoptosis in
response to some triggers. Pkr is itself a candidate “death gene”
in which overexpression causes apoptosis (28). This has been
shown to require the third basic region implicated in Pkr autori-
gulatory regulation (53). Pkr is located upstream of
Bcl-2 function since Bcl-2 abrogates Pkr-mediated apoptosis (27).
Expression of inactivated Pkr suppresses influenza-medi-
ated apoptosis (26) and antisense ablation of Pkr provides
immunity to TNF-α (36).
Both viral growth and virus-induced apoptosis is normal in
Pkr−/− cells further supporting the idea that the biological roles
of Pkr are assisted by parallel pathways. Recently, Taniguchi
et al. (20, 25) may be accounted for by mouse strain differences. The
defect in erythropoietin-induced erythroid CFU development may in-
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