Characterization of Two Evolutionarily Conserved, Alternatively Spliced Nuclear Phosphoproteins, NFAR-1 and -2, That Function in mRNA Processing and Interact with the Double-stranded RNA-dependent Protein Kinase, PKR*

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We report here the isolation and characterization of two proteins, NFAR-1 and -2, which were isolated through their ability to interact with the dsRNA-dependent protein kinase, PKR. The NFAR proteins, of 90 and 110 kDa, are derived from a single gene through alternative splicing and are evolutionarily conserved nuclear phosphoproteins that interact with double-stranded RNA. Both NFAR-1 and -2 are phosphorylated by PKR, reciprocally co-immunoprecipitate with PKR, and colocalize with the kinase in a diffuse nuclear pattern within the cell. Transfection studies indicate that the NFARs regulate gene expression at the level of transcription, probably during the processing of pre-mRNAs, an activity that was increased in fibroblasts lacking PKR. Subsequent functional analyses indicated that amino acids important for NFAR's activity were localized to the C terminus of the protein, a region that was found to specifically interact with FUS and SMN, proteins also known as regulators of RNA processing. Accordingly, both NFARs were found to associate with both pre-mRNAs and spliced mRNAs in post-transcriptional studies, similar to the known splicing factor ASF/SF-2. Collectively, our data indicate that the NFARs may facilitate double-stranded RNA-regulated gene expression at the level of post-transcription and possibly contribute to host defense-related mechanisms in the cell.

The interferons (IFNs)† are pleotropic cytokines, considered important in host defense, that manifest their biological properties by inducing a number of responsive genes (1). One key IFN-inducible gene is the serine/threonine, dsRNA-dependent protein kinase, PKR, with a molecular mass of 68 kDa in human cells (2). PKR is a member of a previously described family of evolutionarily conserved dsRNA binding molecules that includes Escherichia coli RNase III, Drosophila staufen, and Xenopus 4F.1 (3, 4). Interaction with dsRNA structures of greater than 35 bp causes PKR to autophosphorylate and subsequently to catalyze the phosphorylation of substrate targets, the most well characterized being the α subunit of eukaryotic initiation factor eIF2α (5–7). Phosphorylated eIF2α effectively sequesters eIF2B, a rate-limiting component of translation, and causes a dramatic inhibition of protein synthesis in the cell (8, 9).

The majority of studies relating to PKR's function have focused on the kinase's participation in IFN's retaliatory response to virus infection. For example, PKR can be activated by a number of virus specific RNAs (10–12). PKR-deficient mice are extremely sensitive to lethal pulmonary disease following infection with vesicular stomatitis virus or influenza virus, further emphasizing the importance of PKR in host defense (13). Since activation of PKR is highly detrimental to the virus life cycle, numerous viruses have evolved mechanisms to neutralize the function of this kinase. For example, vaccinia virus encodes two proteins capable of inhibiting PKR. The first of these products, referred to as E3L, contains a dsRNA binding domain similar to those found in PKR (14–16). E3L, which is primarily detected in the nucleus, competes for dsRNA activators and may even bind to and inhibit the kinase through dsRNA bridging (17). A second vaccinia protein, K3L, shares homology to the known PKR substrate eIF2α and is thought to function by competitively sequestering the kinase (15, 18, 19). In addition, adenovirus may utilize VAI RNA to suppress PKR, while hepatitis C has been reported to encode PKR-interacting proteins referred to as NS5a and E2 (20–23).

Recent data have also revealed that PKR almost certainly plays a role in signaling events in the cell, including dsRNA-induced apoptosis. For example, recombinant vaccinia viruses overexpressing wild-type PKR have been shown to induce the rapid apoptosis of HeLa cells (16, 24, 25). Murine fibroblasts deficient for PKR activity have also been reported to be more resistant to dsRNA and tumor necrosis factor-α-induced apoptosis (26, 27). Our laboratory has recently demonstrated that cells inducibly overexpressing functional PKR, but not a dominant-negative PKR variant, are sensitive to dsRNA-mediated and virus-induced cell death (28). PKR-dependent apoptosis was accompanied by the induction of Fas, a member of the tumor necrosis factor receptor family, and was mediated...
through the FADD death-signaling pathway (28). Although the mechanisms of PKR-induced apoptosis remain to be fully clarified, it has been reported that PKR may play a role in the induction of NF-κB, a key regulator of cell survival, in response to viral infection and dsRNA. For example, murine fibroblasts lacking PKR exhibit diminished responses to dsRNA signaling as indicated by reduced activity of NF-κB and IRF-1 (29). Although presently controversial, this regulation may involve PKR influencing the activity of the IκB kinase (IKK) complex leading to the autophosphorylation of IKK and degradation of IκB (30, 31). In addition to these roles, PKR may also be involved in signaling pathways involving Ras, interleukin-3, and growth factors such as platelet-derived growth factor (32). Collectively, these data strongly suggest that substrates of PKR other than eIF2α probably exist in the cell, although their identification and characterization have thus far remained elusive.

Accordingly, to identify possible regulators and/or substrates of PKR, we used the yeast two-hybrid system to isolate proteins that associate with this kinase. Here we report the isolation and characterization of two evolutionarily conserved, dsRNA-binding nuclear proteins, NFAR-1 and -2 (for nuclear factors associated with dsRNA). This work confirms that the NFAR gene on chromosome 19 gives rise to two alternatively spliced variants and correctly identifies the two NFAR proteins. Aside from demonstrating that the NFARs associate with PKR in yeast, we show that both NFAR-1 and -2 share homology with eIF2α, the known PKR cytoplasmic substrate, and are themselves substrates for PKR. In transfected cells, both NFARs were found to regulate the transcription of co-transfected reporter genes, probably at the level of mRNA elongation, an effect that was increased in PKR null fibroblasts. Transfection studies further indicated that NFAR-2 rather than NFAR-1 appeared to have more potent regulatory activity and that the C-terminal region unique to NFAR-2 exhibited constitutive activity and interacted with SMN and FUS, two proteins known to play a role in mRNA processing. Finally, we report that NFAR can associate with active splicesomes. Our data indicate that the NFARs are evolutionarily conserved mediators of gene expression that may function as proximal targets for PKR and may play a role in regulating gene expression in response to dsRNA-regulated signaling events in the cell.

**EXPERIMENTAL PROCEDURES**

**Plasmids—**Construction of PKR K296R in pET11a (Novagen, CA) has been previously described (33). To fuse the GAL4 DNA binding domain in frame with PKR, the EcoRI site in pGTE9 (CLONTECH) was mutagenized to an Ndel site to create pGTT10. Full-length PKR K296R was digested with Ndel and BamHI and then inserted into similarly treated pGBT10, generating pGBT DII.

The full-length CDNs of NFAR-1 and NFAR-2 were generated by subcloning C terminus PstI-BglII NFAR fragments from yeast two-hybrid pGAD10 vectors into pGAD424 (CLONTECH). The N terminus of NFAR was amplified by PCR from cDNAs isolated screening a Jurkat T-cell library and was joined to the C terminus of NFAR at an endogenous PstI site. An Ndel site was engineered into the N terminus of NFAR during PCR. For bacterial expression, Ndel-BglII-digested NFAR-1 and -2 from pGAD424 were subcloned into pET14b digested with Ndel and BamHI (Novagen). For mammalian cell expression, NFAR cDNAs from pGAD were digested with EcoRV, followed by treatment with Klenow fragment and dNTPs to blunt the N terminus. NFAR was then subsequently digested with BglII and subcloned into EcoRV-BamHI-digested pcDNA3 (Invitrogen). To fuse the C terminus of NFAR-2 to the Gal4 binding domain, amino acids 666–894 of NFAR-2 were amplified by PCR using NFAR-2 as a template and subcloned into the EcoRI and PstI sites of pGBT9.

NFAR-2 deletion mutations M5 (∆77, 173), M7 (∆174–232), M8 (∆238–384), M10 (∆1–369), M11 (∆1–588), and NFAR-1 M10 (∆1–369) were created in pET14b and pcDNA3.1 vectors using the Stratagene QuickChange site-directed mutagenesis kit. NFAR-2 M12 (∆586–894) and NFAR M13 (∆1–369, 586–894) were created by digesting the constructs with HindIII, which cuts at an endogenous site in NFAR after the dsRNA binding domains, and in the MCS of pcDNA3.1 and then religating the vector to remove the C terminus of NFAR-2. The NFAR M9 variant (amino acids 1–417) was created by cloning the NFAR NCRRI fragment into pGAD424 and subsequently into pET11 and pcDNA3.1. All NFAR mutants were confirmed by DNA sequencing.

**Yeast Strains and Two-hybrid Screen—**PKR DII and NFAR (amino acids 666–894) in pGBT9 were used to transfect the yeast strains Saccharomyces cerevisiae Y190 and PJ6944, respectively, as described (CLONTECH).

**Histidine-tagged Fusion Protein Expression and Purification—**PKR was cloned into pET11a such that the amino terminus of the kinase was fused in frame with 6x histidine residues (Novagen). The resultant plasmid (pET14b-PKR) was used to transform E. coli BL21(DE3)pLysS (Novagen), and PKR was purified over nickel columns as previously described (34). His-tagged proteins were further purified on a gel filtration column, Superose 12 (Amersham Pharmacia Biotech), examined by Coomassie Blue-stained SDS-polyacrylamide gels or immunoblot using anti-PKR monoclonal antibody (35).

For expression of 35S-labeled NFAR in vitro, the TNT coupled transcription/translation system (Promega) was used to synthesize proteins from the pcDNA3.1 NFAR vectors using T7 polymerase.

**dsRNA Binding Assays—**The dsRNA binding activity of NFAR was measured by incubating purified His-tagged NFAR proteins, 35S-labeled NFAR proteins, or lysate from HeLa cells with poly(I-C)-agarose beads. The HeLa cells were lysed in binding buffer (20 mM Tris-HCl, pH 7.5, 0.2 mM PMSF, 100 μg/ml aprotinin, and 6 M urea) and dialyzed overnight (20 mM Tris-HCl, pH 8.0, 50 mM NaCl, 0.1 mM EDTA, 0.2 mM PMSF, 100 μg/ml aprotinin, and 10% glycerol) to remove the denaturing reagent. Purified His-tagged NFAR proteins were examined by Coomassie Blue-stained SDS-polyacrylamide gels and by immunoblot using a monoclonal antibody to the His tag (R & D Systems).

For expression of 35S-labeled NFAR in vitro, the TNT coupled transcription/translation system (Promega) was used to synthesize proteins from the pcDNA3.1 NFAR vectors using T7 polymerase.

**Kinase Assays—**For in vitro assays, purified PKR was incubated with approximately stoichiometric amounts of His-tagged NFAR-1 or -2 in equal volumes of KCl buffer (20 mM Tris-HCl, pH 7.4, 25 mM KCl, 1 mM dithiothreitol, 100 mM PMSF, 1 μg/ml aprotinin, and 0.5% glycerol) in the presence or absence of dsRNA as described (28).

**Northern Blot Analysis—**Adult and fetal human multiple tissue Northern blots (CLONTECH) were hybridized with radiolabeled cDNA representing NFAR-1. Blots were washed and autoradiographed prior to being reprobed with radiolabeled actin cDNA.

**Co-immunoprecipitation Assays—**For in vitro analysis, equal amounts of His-tagged PKR and NFAR-1 were incubated in binding buffer (0.2% Nonidet P-40, 80 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, 0.1 mM PMSF, 1 μg/ml aprotinin, and 5% glycerol) in the presence or absence of dsRNA as described (28).

**Confocal Microscopy—**HeLa cells grown on coverslips were fixed at room temperature in freshly prepared 2% paraformaldehyde in 1× PBS and permeabilized for 60 s with 0.1% Triton X-100 in 1× PBS and 0.1 mM glycine. Antibodies used were anti-PKR (33), anti-SMN (Transduction Laboratories), and hybridoma supernatant of anti-FUS monoclonal antibody (gift from David Ron) followed by anti-mouse secondary antibody conjugated with Texas Red (Molecular Probes, Inc., Eugene, OR). Cells were double-labeled with anti-NFAR rabbit polyclonal serum and then rabbit secondary antibody conjugated with fluorescein isothiocyanate (Molecular Probes).

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Characterization of PKR Substrates, NFAR-1 and -2

RESULTS

Isolation and Identification of NFAR-1 and -2—To identify novel proteins that interact with PKR, we used the yeast two-hybrid system and screened for proteins that associated with the full-length kinase (38). Wild-type PKR is toxic to yeast, since it becomes activated in vivo and phosphorylates the yeast eIF2α homologue, SU122, causing a block in translation (39, 40). Therefore, a catalytically inactive PKR variant with an amino acid substitution in catalytic domain II (K296R) was fused to the Gal4 DNA binding domain and used as bait to screen a Jurkat T-cell cDNA library. Eight positive clones from 4 × 10⁶ transformants screened were found to be identical at the N terminus of the putative open reading frames but to differ in their carboxyl ends, indicating the existence of two related gene products, which we refer to as NFAR-1 and -2. Further isolation of cDNA clones retrieved from hybridization screening of T-cell cDNA libraries and the complete sequencing of the entire NFAR gene from PAC clones confirmed the existence of two related cDNAs that are generated through alternative splicing from a single gene on chromosome 19 (Fig. 1A and B; Ref. 41). The two NFAR cDNAs represented open reading frames encoding proteins containing 702 (NFAR-1) and 894 (NFAR-2) amino acids, respectively. The coding region of both alternatively spliced variants begins in exon 2 and encodes through exon 17, at which point NFAR-1 continues to encode one additional exon, exon 18, while NFAR-2 splicees to exon 19 and encodes three additional exons not contained in NFAR-1 (Fig. 1B).

Data base searches revealed that amino acids 401–468 and 524–591 of both NFAR-1 and -2 represent potential dsRNA binding motifs, similar to those found in PKR (4, 42). Thus, NFAR-1 and -2 are almost certainly members of the dsRNA binding family of proteins that includes the Drosophila maternal effect protein staufen and the vaccinia virus protein E3L (4). Data bank comparative analysis further revealed that NFAR-1 and -2 are ~75% homologous to the amino acid acid level with the Xenopus dsRNA-binding proteins 4F.1 and 4F.2, whose function is unknown (3). Significantly, both NFAR proteins were also found to share homology from amino acids 166–235 with the known PKR substrate eIF2α (Fig. 1C; 23% identity, 49% conservation over 69 amino acids). It is notable that this homologous region of eIF2α contains serine 51, the single target site for PKR phosphorylation (9). More extensive regions of homology exist from residue 222 to 235 and include a conserved VIIR box. This area also entirely overlaps with the vaccinia virus-encoded PKR inhibitor, K3L, an eIF2α homologue (15, 18). It has been reported that K3L binds 400-fold more efficiently than eIF2α to PKR and suppresses the function of the kinase (19).

The sequences of the NFAR cDNA clones were homologous to two partially overlapping segments in the data banks, referred to as MPP-4 (43). However, the two MPP-4 clones are partial representations of the NFAR gene with the coding regions of these genes only extending to amino acids 404 and 611 of NFAR-1. Our sequence was also similar (85% identity at the amino acid level) to a protein referred to as NF-90 (44). However, careful comparison of the NFAR-2 cDNA sequence indicates that 2 bp have been inserted into the NF-90 sequence (nucleotides 1798 and 1799 of the NF-90 coding region), producing a shift in the reading frame to generate a nonnatural hybrid protein. A recent isolate, DRBP76, with unknown function, appears to be 99% identical to NFAR-1 (45), while the human NFAR-2 gene does not appear to have been previously isolated.

In Vivo Expression and Tissue Distribution of NFAR-1 and -2—To start to characterize the expression profile of the NFARs, selected cell lines were examined by immunoblot anal-

noprecipitate NFAR, rabbit antiserum raised to NFAR-1 or control preimmune rabbit serum was added to the proteins as above. After washing, the proteins were electrophoresed as described and immunoblotted using anti-PKR monoclonal antibody.

For in vivo co-immunoprecipitation analysis, HeLa cells (3 × 10⁶ cells) were plated out in 12-well dishes for luciferase assays, 0.7 µg of the pcDNA1 vector and 0.3 µg of the pGL3 promoter vector were co-transfected using the LipofectAMINE Plus reagents (Life Technologies, Inc.). Cells were lysed 48 h post-transfection in 10 µl of lysis buffer (20 mM Tris-HCl, pH 7.5, 50 mM KCl, 1 mM NaCl, 1% Nonidet P-40, 1 mM EDTA, and 0.2 mM PMSF). Luciferase assays were read on a luminometer (TD 20/20; Promega). All assays were performed independently, a minimum of three times, in duplicate. Mice lacking PKR (strain 129/tcrsv) were courtesy of Dr. John Bell of Ontario University (36).

RT-PCR—COS-1 cells were transfected as above, except that 1 × 10⁵ cells were seeded into six-well dishes and transfected with 1 µg of the pcDNA3.1 vector and 0.4 µg of the reporter vector. Total RNA was isolated using TRIzol (Life Technologies) according to the manufacturer’s protocol. After quantitation, the samples were treated with DNase I (Life Technologies). RT-PCR was performed using Stratagene’s Prot-TAR cDNA synthesis kit with an input of 10⁷ COS-1 cells. First strand cDNA synthesis was performed in 20 µl of reaction volume containing 5 units of RNase T1 (Roche Molecular Biochemicals) was added during the third wash and incubated at 4 °C for 15 min. The proteins were examined by immunoblot using anti-PKR, anti-NFAR, anti-FUS, or anti-SMN antibodies.

Nuclear Run-on Assay—NFAR-1 or NFAR-2 was co-transfected with pGL3 promoter (Promega) into 100 mm dishes of 1 × 10⁵ COS-1 cells. At 36 h post-transfection, the cells were washed three times in ice-cold 1× SSC, collected, and resuspended in 100 µl of Nonidet P-40 lysis buffer (10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 3 mM MgCl₂, 0.1 mM EDTA, 0.5% Nonidet P-40) by pipetting 10 times. Cells were incubated on ice for 5 min, centrifuged, and washed twice in 1 ml of the above buffer and then resuspended in 100 µl of freezing buffer (50 mM Tris-HCl, pH 8.3, 40% glycerol, 5 mM MgCl₂, 0.1 mM EDTA) and frozen in liquid nitrogen. The nuclei were thawed on ice and mixed with 100 µl of freezing buffer (50 mM Tris-HCl, pH 8.0, 5 mM MgCl₂, 300 mM KCl, 0.5 mM each ATP, CTP, and GTP, and 100 µl of α-32PUTP, 800 Ci/mmol). Following a 20-min incubation at 30 °C, 10 mg/ml DNase I was added, and the nuclei were incubated at 42 °C for 30 min. Labeled RNAs were isolated using the GeneClean II (Bio101) kit. cDNA synthesis was then performed in 6 × SSC on ice. The cDNAs were applied to microtiter plates for hybridization.

Immunoprecipitation of Spliceosomes—Immunoprecipitations of in vitro splicing reactions using NFAR-specific antisperm and other control antibodies were performed as previously described (37). Anti-heterogeneous nuclear ribonucleoprotein A1 monoclonal antibody (9H10) was a generous gift from Dr. G. Dreyfuss.
ysis using a novel rabbit antiserum raised in our laboratory to
E. coli purified His-tagged NFAR-1. Fig. 2A confirmed that
NFAR exists as two major species with approximate molecular
masses of 90 and 110 kDa in the cells examined, including
Jurkat and HeLa cells. To determine if the isolated NFAR
cDNA clones encoded similar sized proteins, His-tagged
NFAR-1 and -2 cDNAs were expressed transiently in mammalian cells (Fig. 2B). Immunoblot analysis of transfected 293T cell lysates precipitated with poly(I-C) and immunoblotted with anti-NFAR antiserum indicated that the recombinant NFAR-1 migrated identically to the 90-kDa protein observed in 293T cells probed with NFAR antisera, while recombinant NFAR-2 migrated similarly to the 110-kDa product. Taken together, these observations strongly indicate that the cloned NFAR cDNAs are full-length representations of the major NFAR species of 90 and 110 kDa that exist in mammalian cells.

To extend our analysis of the expression profile of the NFAR genes, mRNA and protein were examined from various human tissues. Northern blot analysis indicated that NFAR mRNAs were transcribed in nearly all of the human tissue examined (Fig. 2C). Although our antiserum indicated the existence of only two major NFAR polypeptides, we found that a number of NFAR-related mRNA species were apparent (4–8 kb). Thus, a variety of spliced variants of the NFAR gene could exist in the cell, or our radiolabeled probe could cross-react with other dsRNA-binding proteins. To check the translational status of these transcripts, representative proteins from tissue analyzed by Northern blot were examined by immunoblot analysis using NFAR antiserum (Fig. 2D). In the majority of tissues examined, the two major species of NFAR, namely the 90- and 110-kDa proteins, were visible. Interestingly, lesser amounts of NFAR were seen in the liver and spleen and to a certain extent kidney, suggesting that the regulation of these proteins may be governed to some extent at the translational or post-translational level.

Since the NFARs showed extensive homology to Xenopus 4F.1 and 4F.2, we next examined whether antiserum raised to human NFAR could cross-react with Xenopus proteins. Xenopus oocytes and embryos at various stages of development were therefore lysed, precipitated with poly(I-C)-agarose, and immunoblotted using our human anti-NFAR serum. Fig. 2E shows that the antiserum detected two poly(I-C)-binding proteins with molecular masses of 95 and 115 kDa in all extracts examined. Furthermore, similar sized dsRNA-binding proteins were observed in insect Sf9 cells. These data strongly suggest that the proteins detected in Xenopus and insect cell extracts are homologues of NFAR-1 and -2 and that both alternatively spliced proteins have been conserved through evolution.

NFAR-1 and -2 Are dsRNA-binding Proteins—To demonstrate that the NFAR proteins are able to interact with dsRNA, poly(I-C)-agarose beads or agarose beads alone were used to precipitate endogenous NFAR-1 and -2 from extracts of HeLa cells. Complexes were then washed under various salt conditions, electrophoresed, and immunoblotted using NFAR antiserum. Fig. 3A shows that both NFAR-1 and -2 bound efficiently to poly(I-C)-agarose beads but not to agarose beads alone. Increasing the salt concentration to 1 M NaCl prevented the binding of the NFAR proteins to the beads. Furthermore, purified recombinant NFAR-1 and -2, expressed in E. coli (His-NFAR-1 and -2), could also bind to poly(I-C)-agarose beads but not to agarose beads alone.

To confirm that the dsRNA binding motifs were responsible for the interaction with dsRNA, a number of NFAR variants were generated using a rabbit reticulocyte, in vitro coupled
400 mM NaCl, the proteins were electrophoresed (gel and electrophoresed). Equal amounts of NFAR protein were incubated with poly(I-C)-agarose beads, and after extensive washing in top panel were generated using an incubation under various salt conditions (0–1 M NaCl) with poly(I-C)-agarose or agarose alone and treated similarly. B, schematic diagram of NFAR variants used in dsRNA binding experiments. NFAR variants were generated with the following amino acids deleted: NFAR-2 M5 (Δ157–173), NFAR-2 M7 (Δ174–232), NFAR-2 M8 (Δ238–384), NFAR M9 (Δ418–702), NFAR-1 and -2 M10 (Δ1–369), NFAR-2 M11 (Δ1–588), NFAR-2 M12 (Δ586–894), and NFAR M13 (Δ1–369, 586–894). C, the dsRNA binding motifs of NFAR are necessary for the interaction with dsRNA. 35S-Labeled NFAR proteins were generated using an in vitro coupled transcription and translation system. Equal amounts of protein were loaded on an 8.5% polyacrylamide gel and electrophoresed (top panel). Equal amounts of NFAR protein were incubated with poly(I-C)-agarose beads, and after extensive washing in 400 mM NaCl, the proteins were electrophoresed (bottom panel). IP, immunoprecipitate.

transcription and translation system (Fig. 3B). Equal amounts of the various His-tagged NFAR proteins (Fig. 3C, top panel) were incubated with poly(I-C)-agarose and washed with high salt buffer (Fig. 3C, bottom panel). Only the NFAR constructs containing both dsRNA binding motifs were able to bind poly(I-C)-agarose, while NFAR M9 and NFAR-2 M11, which lack both dsRNA binding domains, failed to bind dsRNA (Fig. 3, B and C). Thus, we confirm that the region of the NFARs that contain both dsRNA binding motifs is essential for the interaction with dsRNA.

PKR and NFAR Reciprocally Co-immunoprecipitate and Co-localize in the Nucleus of the Cell—To further analyze the association of the NFARs with PKR, we determined whether NFAR-1 and -2 could co-immunoprecipitate with PKR. E. coli expressed and affinity-purified His-tagged PKR and NFAR-1 or NFAR-2 were incubated in vitro and co-immunoprecipitated using an anti-PKR monoclonal or isotyped control antibody. Extracts were treated with a variety of RNases to eliminate potential dsRNA bridging between the proteins. Immunoblotting with the NFAR antiserum revealed that both NFAR-1 and NFAR-2 associated with PKR when both recombinant proteins were included in the immunoprecipitation reaction (Fig. 4A, right panel). Similarly, PKR was found to reciprocally co-immunoprecipitate with NFAR-1 and -2 when NFAR antiserum was used only when both recombinant proteins were included (Fig. 4A, left panel).

To demonstrate the interaction of the NFARs and PKR in vivo, untransfected HeLa cell extracts, treated with RNases, were incubated with NFAR antiserum. As shown in Fig. 4B, endogenous PKR co-immunoprecipitated with endogenous NFAR-1 and -2 from HeLa cells as confirmed using anti-PKR monoclonal antibodies in immunoblot analysis (Fig. 4B, left panel). PKR was not detected when preimmune rabbit serum was used in the immunoprecipitation procedure. Likewise, both NFAR-1 and -2 co-immunoprecipitated with PKR when the anti-PKR monoclonal antibody was used in the precipitation procedure but not when isotyped control antibody was used (Fig. 4B, right panel). Taken together, our data strongly indicate that PKR associates with NFAR-1 and -2 not only in vitro but also in vivo.

PKR is predominantly found in the cytoplasm, where it is closely associated with ribosomes (46, 47). However, electron micrograph analysis and immunogold labeling has also indicated that a fraction of PKR resides in the nucleus (48, 49). Since the NFARs have a consensus bipartite nuclear localization signal (Fig. 1, A and B), we determined if PKR colocalizes with NFAR in the cell. We employed confocal microscopy double labeling studies using rabbit polyclonal and murine monoclonal antibodies directed to NFAR and PKR, respectively. HeLa cells incubated with both antibodies revealed that the distribution of NFAR is predominantly in the nucleus (Fig. 4C, middle panel). However, although the majority of PKR was found in the cytoplasm, a fraction (~20–25%) was diffusely distributed in the nucleus (Fig. 4C, left panel). Importantly, overlaying the images revealed that NFAR and PKR showed evidence of colocalization in the nucleus of the cell (Fig. 4C, right panel).

NFAR-1 and -2 Are Nuclear Phosphoproteins and Substrates for PKR—Sequence analysis revealed that the NFAR proteins exhibited homology with the known PKR substrate eIF2α (Fig. 1C). To determine if the NFAR proteins are indeed substrates for PKR, NFAR-1 and -2 were expressed as His-tagged proteins in E. coli and purified over nickel affinity columns to ~90% purity as estimated by Coomassie Blue-stained gels (data not shown). PKR was also expressed as a His-tagged protein and underwent a similar purification protocol followed by a gel filtration step. Our data indicated that NFAR-1 or -2 alone showed no indication of autophosphorylation when incubated.
in the presence of dsRNA and [γ-32P]ATP (data not shown). In contrast, PKR efficiently autophosphorylated when incubated with dsRNA, as has been previously described (Fig. 5A, left panel). At high levels of dsRNA, PKR autophosphorylation is typically inhibited, arguably since intermolecular PKR-PKR interactions are prevented by each PKR molecule individually (50–52). Significantly, PKR was clearly able to phosphorylate stoichiometric amounts of both NFAR-1 as well as NFAR-2 when incubated together (Fig. 5A, middle and right panels). At higher concentrations of dsRNA, phosphorylation of NFAR-1 and -2 by PKR was inhibited, similar to the effect seen with PKR alone.

To examine the role of the dsRNA binding domains in the interaction between PKR and NFAR, an NFAR variant lacking both dsRNA binding motifs (NFAR M9, amino acids 1–415; Fig. 3C) was next examined as a substrate for the kinase. Fig. 5B demonstrates that NFAR M9 that lacks the dsRNA binding domains was also able to serve as an efficient substrate for PKR. These data suggest that dsRNA is not essential for the association of the two proteins and emphasizes that PKR phosphorylates a site(s) in the N terminus of NFAR that includes the region with homology to eIF2α.

Attempts at comparing NFAR phosphorylation levels in PKR null and control mouse embryonic fibroblasts (MEFs) illustrated a high basal level of phosphorylation, probably due to several kinases regulating NFAR activity (data not shown). To start to evaluate whether the NFAR proteins can be phosphorylated in vivo by PKR, control of 3T3 L1 cells inducibly overexpressing PKR were treated with poly(I-C) in the presence of [γ-32P]orthophosphate (28), followed by precipitation of NFAR and PKR using poly(I-C)-agarose beads. Fig. 5C confirmed that at least a proportion of the NFARs exist as phosphoproteins in vivo. Significantly, 3T3 L1 cells overexpressing the heterologous PKR (WT-PKR) severalfold higher than the endogenous murine PKR in the control cells (VEC) contained 3-fold higher levels of phosphorylated NFAR-1 and -2 (Fig. 5C, right panel). Immunoblotting equal amounts of the poly(I-C)-precipitated protein complexes using NFAR antiserum (Fig. 5C, left panel) confirmed the identity of the NFARs and ensured that the level of the NFAR proteins was equal in both the control and PKR overexpressing cells (demonstrating that the increase in NFAR phosphorylation is not a reflection of elevated NFAR protein levels). Taken together, these data imply that the NFARs exist as nuclear phosphoproteins and are conceivably substrates for PKR in vivo.

**NFAR-1 and -2 Regulate Gene Expression at the Level of Transcription**—To begin to examine a possible role of the NFARs in the regulation of gene expression, NFAR-1 and -2 were co-transfected into COS-1 cells with a luciferase reporter gene driven by the SV40 promoter. Fig. 6A indicates that luciferase production was augmented in the presence of NFAR-1 (~2-fold) and NFAR-2 (~3-fold), signifying that both
NFARs can indeed influence the regulation of gene expression. To further examine NFAR’s function, several NFAR variants were similarly examined for their ability to modulate gene expression. All NFAR constructs were His-tagged, and immunoblots were carried out on transfected cells to ensure equal expression of the heterologous proteins (Fig. 6B). Deletion of various regions in the N terminus of NFAR-2, namely NFAR M5 (∆7–173), NFAR M7 (∆174–232), and NFAR M8 (∆238–384), did not significantly impair the ability of NFAR-2 to stimulate luciferase synthesis (Figs. 3B and 6A). However, deletion of the first 269 N-terminal amino acids from either NFAR-1 or -2 (NFAR-1 and -2 M10) did appear to increase NFAR’s capability to regulate gene expression, suggesting that the C terminus of the protein may function as a constitutively active form of the NFARs. This data was complemented by showing that the amino terminus region of the NFARs (NFAR M9), lacking the active C-terminal portion of the molecule, exhibited greatly reduced function (Figs. 3B and 6A). However, NFAR-2 M12, in which the protein terminates after the dsRNA binding domains, and NFAR-2 M11 and M13, which contain the dsRNA binding motifs or only the C terminus region, respectively, were also found to be impaired in their ability to manipulate luciferase synthesis (Figs. 3B and 6A). Thus, our data indicate that both the dsRNA binding motifs and C-terminal portion of the NFARs are required for optimal NFAR function.

To determine whether the regulation of gene expression was governed at the transcriptional or translational level, cells were co-transfected with NFAR-1 or -2 and a luciferase reporter gene driven by the SV40 promoter. Total RNA was isolated after 48 h, and RT-PCR measured luciferase mRNA levels as well as actin mRNA levels to ensure that approximately equal amounts of RNA were analyzed. Fig. 6C demonstrates increases (>2-fold) in the level of reporter gene transcripts in cells transfected with NFAR-1 or -2 but not vector alone, suggesting that the NFARs function to regulate transcription. Conceivably, the NFARs could function at the initiation, elongation, or post-transcriptional stage of mRNA production. However, further investigations using highly purified NFAR proteins in EMSAs indicated that the NFARs do not appear to bind directly to SV40 promoter sequences (data not shown). Furthermore, no transcription activation activity was apparent in co-transfection experiments with reporter genes under control of the Gal4 promoter and NFAR-1 or -2 fused to a Gal4 binding domain (data not shown). To examine if NFAR influences mRNA stability, we again co-transfected COS-1 cells with NFAR and the luciferase reporter construct, and 24 and 48 h after co-transfection the cells were treated with actinomycin D. Luciferase levels were then measured at several intervals up to 55 h, and NFAR did not appear to affect the stability of mRNA (data not shown). Collectively, these data strongly suggest but do not entirely validate that the mechanisms of NFAR action are downstream from the initiation of transcription.

To further elucidate at which step of transcription/post-transcription the NFARs function, nuclear run-on assays were performed. Accordingly, NFAR-1 and -2 were co-transfected in COS-1 cells along with an SV40 luciferase reporter construct,
FIG. 6. The NFARs function to regulate gene expression. A, NFAR-1 and -2 stimulate the expression of co-transfected reporter genes under control of the SV40 promoter. Equal quantities of eukaryotic expression vectors (pcDNA3.1) carrying NFAR-1 or -2 or variants were co-transfected in COS-1 cells with a luciferase reporter gene placed downstream of the SV40 promoter (pGL3 promoter). After 48 h, the cells were lysed, and luciferase production was assayed. Experiments were done in duplicate three times. The mean values of the experiments are shown. B, immunoblot of NFAR variants transiently expressed in COS-1. NFAR-1 and -2 and NFAR variants (Fig. 3B) were transfected into COS-1 cells. After 48 h, NFAR protein expression was monitored by lysing the cells and immunoblotting with a histidine-specific antibody (His-G; Invitrogen). C, the NFARs are regulators of transcription in the cell. NFAR-1 and -2 were co-transfected in COS-1 cells with luciferase reporter genes under control of the SV40 promoter. After 48 h, the cells were lysed, and total RNA was extracted. mRNA was quantitated by RT-PCR using probes to actin as described in Experimental Procedures. D, Luciferase mRNA was measured using primers specific to the luciferase gene. The NFARs enhance transcription elongation. A nuclear run-on assay was performed and showed that co-transfected NFAR-1 or -2 increased the density of elongating RNA polymerases at the 3′-end of luciferase ~1.5–2-fold over vector alone. The density of engaged RNA polymerases on GAPDH was measured to control for the amount of RNA on each slot blot. and nuclei were isolated and labeled rNTPs were added to allow elongation of engaged polymerases. To equalize the amount of isolated RNA, GAPDH mRNA was measured along with luciferase. The ratio of GAPDH probe to the N terminus of luciferase showed that neither NFAR-1 nor NFAR-2 increased the rate of transcription over the vector control. However, a probe to the C terminus of the luciferase mRNA indicated that NFAR-1 and -2 did appear to moderately increase the rate of transcription ~1.4- and 2-fold, respectively, compared with vector-transfected (Fig. 6B). Collectively, our data would indicate that the NFARs function at the level of transcription elongation or in the processing of mRNAs.

Analysis of the Regulation of NFAR by PKR—Our yeast genetic studies, co-immunoprecipitation data, confocal analyses, and phosphorylation experiments indicate that PKR may associate with the NFARs in the cell. To examine if PKR influences NFARs' ability to regulate gene expression in vivo, immortalized control and PKR null MEFs were transfected with NFAR-1 or -2 and the SV40 promoter luciferase reporter construct. Immunoblot analysis was carried out on control and PKR null MEFs to demonstrate that endogenous levels of NFAR were equal in both cell types and that PKR is lacking in PKR−/− cells (Fig. 7B). Surprisingly, unlike transfected COS-1 cells, NFAR did not appear to strongly stimulate gene expression in the control immortalized MEFs, perhaps due to cell-specific factors being absent in the MEFs compared with COS-1 cells or due to the fact that COS-1 cells are transformed. However, in PKR null fibroblasts, NFAR-1 was found to stimulate gene expression ~4.0-fold, and NFAR-2 stimulated expression ~3.0-fold, compared with vector alone (Fig. 7A). Although the addition of IFNa/β and poly(I-C), which are known stimulators of PKR expression and activity, respectively, had no effect on NFAR-regulated luciferase expression in the control MEFs, treatment of the PKR null MEFs with poly(I-C) reduced NFAR’s ability to augment gene expression. This effect may be due to the NFARs interacting with other dsRNA binding proteins besides PKR or due to dsRNA binding to the NFARs and inducing a conformational change, which may mask functional domains in the C terminus. Collectively, our data indicate that PKR can modulate the ability of the NFARs to influence gene expression.

NFAR-2 Interacts with the mRNA-processing Proteins SMN and FUS—NFAR-1 and -2 appear to be analogous, alternatively spliced products that have been conserved through evolution, with the only difference between the two being an extra 192 amino acids at the C terminus of NFAR-2. This unique region may allow NFAR-2 to interact with partners not eligible for NFAR-1 and thus to endow extra properties that NFAR-1 lacks. To identify proteins that interact specifically with the C terminus of NFAR-2, amino acids 666–894 were fused in frame to the Gal4 binding domain and used to screen a Jurkat cDNA library in a yeast two-hybrid screen. Full-length cDNA clones were retrieved that encoded proteins able to specifically interact with the C terminus of NFAR-2 (Fig. 8A). One of the interacting clones was identified as SMN (survival of motor neurons), a protein that has been implicated in pre-mRNA splicing and in causing spinal muscular atrophy (53). Interestingly, neither full-length NFAR-1 nor 2 bound efficiently to SMN in yeast studies, typically due to the Gal4 binding domain or the amino terminus region of the NFARs masking the sites responsible for interaction. SMN has been reported to be localized in the cytoplasm and in nuclear bodies called gems, which are adjacent to snRNP-rich coiled bodies (54, 55). Confocal analysis on untransfected HeLa cells using anti-SMN and anti...
NFAR antiserum confirmed that SMN was predominantly localized to nuclear gems, although it also indicated that SMN showed evidence of colocalization with the NFARs in the nucleus (Fig. 8B).

A second protein found to specifically interact with the C terminus of NFAR-2 was FUS/TLS (transformed in liposarcomas), whose N-terminal transactivation domain is fused to the DNA binding domain of CHOP in human myxoid and round cell liposarcomas (56, 57). The C terminus of FUS has conserved RNP consensus sequences as well as RGG repeats, both of which are implicated in RNA binding. Similar to SMN, FUS has been demonstrated to be involved in RNA splicing and/or shuttling of mRNAs as well as in homologous DNA pairing and recombination (58–61). As shown in Fig. 8C, the majority of FUS was localized to the cytoplasm, with a fraction found in a diffuse pattern in the nucleus, where it was also found to colocalize with the NFARs.

To confirm an in vivo interaction between NFAR, FUS, and SMN, HeLa cell lysates were incubated with NFAR, FUS, and SMN, or control antiserum. As Fig. 8D illustrates, NFAR antiserum co-immunoprecipitated both FUS and SMN (top and middle panels). This interaction was not prevented using treatment with RNase T1, indicating that the specificity of the interaction is not simply through RNA bridging. In a reciprocal assay, antibodies to FUS were able to specifically co-immunoprecipitate NFAR-2 but not NFAR-1 (Fig. 8D, bottom panel). This is in agreement with our yeast two-hybrid data that implicate the C terminus of NFAR-2 in the interaction with FUS and SMN. Thus, NFAR-2 but not NFAR-1 is able to interact with proteins such as FUS and SMN, which are known to form...
We used a labeled b-globin to begin to examine a potential role for NFAR in mRNA splicing. Our above data and to further define NFAR’s function, we investigated the properties between the NFARs, these data further indicate the possibility that the NFARs are involved in mRNA processing. Large complexes with snRNP proteins and to be involved in the processing of pre-mRNAs. Aside from indicating divergent properties between the NFARs, these data further indicate the possibility that the NFARs are involved in mRNA processing.

**Immunoprecipitation of NFAR with the Splicesome**—Interaction of the transcription machinery with protein complexes involved in capping, splicing, polyadenylation, and other steps of mRNA processing have been recently described (62). Given our above data and to further define NFAR’s function, we began to examine a potential role for NFAR in mRNA splicing. We used a labeled b-globin pre-mRNA in an *in vitro* splicing reaction and performed an immunoprecipitation with our NFAR antiserum and several other control antibodies. Labeled RNA was recovered from each immunoprecipitated complex, electrophoresed by denaturing polyacrylamide gel electrophoresis, and then detected by autoradiography. As shown in Fig. 9, NFAR preferentially bound to complexes containing spliced mRNAs but was also associated with complexes containing pre-mRNAs as well as intermediate products. This was similar to an immunoprecipitation with an antibody against ASF/SF-2, which is a member of the essential SR protein family, but distinct from that using a control antibody against heterogeneous nuclear ribonucleoprotein A1, which is a regulator of alternative splicing (reviewed in Ref. 63). Collectively, our data suggest that the NFARs may associate with mRNA processing complexes following the initiation of transcription in the cell.

**DISCUSSION**

Using PKR as bait in a yeast genetic screen, we have identified two evolutionarily conserved proteins, NFAR-1 and -2, that are derived through alternative splicing from a single gene on chromosome 19. The NFARs are members of a previously described family of dsRNA-binding proteins that include PKR and vaccinia virus protein E3L (4, 7, 42, 64–66). Our data confirm that both NFAR-1 and -2 are able to interact with dsRNA and establish that the conserved motifs are indeed necessary and sufficient for these interactions (Fig. 3). In addition, using our antiserum to the human NFAR protein, we have identified dsRNA binding proteins that are potential homologs in both insect cells and Xenopus oocytes. Given the availability of a number of NFAR related clones. For example, NFAR-1 is similar to two partial clones called MPP4 and is probably identical to human DRBP76 (99% identical at the amino acid level), which was also reported to be phosphorylated by PKR *in vitro* (43, 45). A recent report further confirms that the existence of a probable mouse homologue of NFAR-2, referred to as mILF3 (91% identical at the amino acid level) and a related human clone, NF-90, whose cDNA sequence is related to NFAR-2 but whose protein appears to be largely artificial, perhaps due to a PCR error introduced during the cloning of the cDNA at the C terminus (41, 44, 67, 68). Collectively, this report represents the first accurate identification and cloning of both alternatively spliced variants of NFAR and confirms that they are derived from a single human gene.

Our data indicate that the NFARs are almost exclusively localized to the nucleus of the cell, and while PKR is known to be distributed predominantly throughout the cytoplasm, this kinase has also been reported to reside in the nucleus (12, 48). Confocal analysis subsequently confirmed that the NFARs and PKR co-localize in a diffuse nuclear pattern within the cell, and co-immunoprecipitation data indicate that the NFARs and PKR could form inactive heterodimers (Fig. 4). It is plausible that dsRNA may facilitate PKR and NFAR interactions by acting as a bridge between two molecules in close proximity, a possibility that has been suggested to occur among other dsRNA binding family members (69). Indeed, data obtained from the yeast two-hybrid system do suggest that dsRNA may facilitate the interaction of the NFARs with PKR (data not shown). Thus, once bound to dsRNA, PKR may preferentially activate nearby targets that also contain dsRNA binding motifs, such as the NFARs.

We further demonstrate that both NFAR-1 and -2 are substrates for PKR (Fig. 5). The phosphorylation of NFAR-1 and -2 was enhanced in the presence of low amounts of dsRNA although inhibited at higher concentrations of dsRNA, speculatively since the dsRNA binding domains of PKR and NFAR may each accommodate an independent RNA molecule (50–52). This may prevent PKR from associating with target substrates such as the NFARs or inhibit the kinase’s catalytic activity. Importantly, an NFAR variant lacking the dsRNA binding domains, NFAR M9, retained its ability to function as a substrate for PKR, indicating that the dsRNA binding domains of the NFARs are not necessary for the interaction with PKR (Fig. 5B). Presently, the residues in the amino region of NFAR-1 and -2 that are specifically targeted for phosphorylation by PKR remain to be resolved. Sequence comparisons have revealed a region of extensive homology between eIF2α, the
viral homologue K3L, and NFAR-1 and -2 (Fig. 1C). Although this homologous area includes a conserved VIR box and the region of eIF2α containing serine 51 that is phosphorylated by PKR, this serine residue is not identically preserved in the NFAR proteins (9, 39). Furthermore, four serine and two threonine residues located within the eIF2α region of homology do not appear to be singularly targeted by PKR for phosphorylation (data not shown). It is possible that this region of homology may facilitate interactions between the NFARs and PKR, similar to the situation between PKR and K3L. Data bank analysis indicates that the NFARs may be targeted by a number of kinases in the cell, since consensus phosphorylation sites for both protein kinase C and casein kinase II are evident. Finally, a recent report also indicates that the partial NFAR clone NF-90 may also be a substrate for DNA-PK (70).

The family of dsRNA-binding proteins has diverse properties in the cell. For example, the adenosine deaminases (ADAR1–3), are interferon-inducible, RNA editing enzymes that catalyze the deamination of adenosine to inosine in dsRNA structures (71–73). In contrast, Drosophila staufen, which has five dsRNA binding motifs, has been shown to play a role in the localization of several mRNAs to specific sites of the developing oocyte and early embryo and in some cases to also activate translation of the specific mRNAs to which it binds (74). The dsRNA-binding proteins, NFAR-1 and -2, that we have identified appear to increase gene expression at the level of transcription elongation and may associate with both pre-mRNA and spliced mRNAs in the cell (Figs. 8 and 9). Through association with splicing proteins, like FUS and SMN, it is plausible that NFAR may be regulated by molecules such as PKR to influence the processing of target mRNAs during viral infection. Interestingly, studies with the NFAR homologues Xenopus 4F.1 and 2 indicate that these molecules were able to associate with RNA/DNA hybrids, also suggesting that they could be associated with a transcriptional elongation/mRNA processing complex (3). However, although the partial NFAR-1 homologue NF-90 was originally identified as binding to ARRE elements in the interleukin-2 promoter, we have been unable to demonstrate that recombiant NFAR proteins or endogenous NFAR bind to these same DNA elements or to the SV40 promoter, this difference perhaps being due to the NF-90 clone lacking an authentic C-terminal region (75, 76).

In addition to NFAR-1 and -2, other dsRNA-binding proteins have been reported to interact with PKR. For example, human SPNR, which has been proposed to have a role in controlling cell growth, has been shown to associate with PKR (69). Further, human TRBP, first isolated by its ability to associate with human immunodeficiency virus TAR RNA, has been documented as being a putative regulator of the kinase (77). In addition, human PACT and its murine homologue RAX have been reported to actually activate PKR and to possibly play a role in stress response (78, 79). Importantly, none of these PKR-interacting proteins have been reported to be substrates for PKR, and to date eIF2α remains the only well documented substrate for this kinase. One possible model to explain the molecular relationship between PKR and NFAR suggests that in the absence of dsRNA, of either cellular or viral origin, PKR becomes activated and is then able to phosphorylate NFAR-1 and -2 to modulate their activity and function. In the case of the SR family of splicing factors, phosphorylation has indeed been shown to be important for regulating the activity of these factors to interact with polymerase II and to form functional splicing complexes (80). Interestingly, PKR has been implicated in influencing the splicing of the tumor necrosis factor-α mRNA, although the exact mechanism remains to be determined (81).

It also remains to be determined why there are two spliced variants of NFAR residing in the cell. NFAR-1 and -2 appear to be expressed concomitantly in nearly all cells and tissue analyzed (Fig. 2), arguing against one spliced form being preferentially translated in certain types of tissue in the absence of the other. It is not yet clear whether the NFARs function as dimers or regulate one another’s activity, although preliminary data argue against NFAR-1 being able to interact with NFAR-2.2 It is possible that the regulation of NFAR-2 may be governed by factors not associated with NFAR-1, since the former protein has an extra 192 amino acids at the extreme carboxyl-terminal end, which is rich in arginine and glycine residues, commonly found in RNA binding and splicing proteins (58). Indeed, yeast two-hybrid screening indicates that the C terminus of NFAR-2 can associate with proteins involved in RNA processing such as FUS and SMN (Fig. 8A). FUS has recently been demonstrated to interact with RNA polymerase II through its N terminus and to interact with members of the SR protein family of splicing factors through its C terminus. Evidence also indicates that FUS may serve as a docking protein that recruits splicing factors to RNA polymerase II, thus coupling gene transcription and splicing (82). In contrast, SMN has been reported to interact with SIP-1 (SMN-interacting protein), forming a large complex containing several U snRNP essential splicing proteins, suggesting a cytoplasmic role for this protein in snRNP biogenesis (83). A nuclear role for SMN in pre-mRNA splicing, probably in the regeneration or recycling of snRNPs and other splicing factors, has similarly been reported (54). That the NFARs were further found to associate with both pre-mRNAs and spliced mRNAs, in vitro, similar to the critical splicing factor ASF/SF-2, further supports a role for the NFARs in RNA splicing/processing (Fig. 9). Collectively, our analyses strongly implicate a role for the NFARs downstream from the initiation of transcription in the cell.

In summary, we have isolated and characterized two alternatively spliced, dsRNA-binding nuclear proteins and provide evidence that the NFARs may function in the processing of mRNA. Our data further indicate that the antiviral protein kinase PKR may interact with NFAR to influence the protein’s function, thus potentially coupling the regulation of post-transcriptional events with host defense mechanisms in the cell.

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Characterization of Two Evolutionarily Conserved, Alternatively Spliced Nuclear Phosphoproteins, NFAR-1 and -2, That Function in mRNA Processing and Interact with the Double-stranded RNA-dependent Protein Kinase, PKR
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