DRBP76, a Double-stranded RNA-binding Nuclear Protein, Is Phosphorylated by the Interferon-induced Protein Kinase, PKR*

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The interferon-induced double-stranded RNA-activated protein kinase PKR is the prototype of a class of double-stranded (dsRNA)-binding proteins (DRBPs) which share a dsRNA-binding motif conserved from Drosophila to humans. Here we report the purification of DRBP76, a new human member of this class of proteins. Sequence from the amino terminus of DRBP76 matched that of the M phase-specific protein, MPP4. DRBP76 was also cloned by the yeast two-hybrid screening of a cDNA library using a mutant PKR as bait. Analysis of the cDNA sequence revealed that it is the full-length version of MPP4, with a bipartite nuclear localization signal, two motifs that can mediate interactions with both dsRNA and PKR, five epitopes for potential M phase-specific phosphorylation, two potential sites for phosphorylation by cyclin-dependent kinases, a RG2 motif present in many RNA-binding proteins and predicts a protein of 76 kDa. DsRNA and PKR interactions of DRBP76 were confirmed by analysis of in vitro translated and purified native proteins. Cellular expression of an epitope-tagged DRBP76 demonstrated its nuclear localization, and its co-immunoprecipitation with PKR demonstrated that the two proteins interact in vivo. Finally, purified DRBP76 was shown to be a substrate of PKR in vitro, indicating that this protein’s cellular activities may be regulated by PKR-mediated phosphorylation.

Among many cellular genes whose transcription is induced by interferons is the protein kinase, PKR (1, 2). This serine/threonine kinase requires activation by autophosphorylation which takes place in the presence of activators such as double-stranded RNA (dsRNA) or heparin. The most well characterized substrate of PKR is the eukaryotic initiation factor eIF-2 (3). Activation of PKR by cellular insults, such as viral infection, causes eIF-2 phosphorylation and concomitant inhibition of protein synthesis. In addition to regulating protein synthesis, PKR affects many other cellular processes including transcriptional signaling (4), apoptosis (5, 6), and cell growth and differentiation (7, 8). Recent evidence has also implicated PKR in cell cycle regulation (9). The identities of the corresponding substrates of PKR that mediate these actions of PKR have remained elusive.

PKR is the prototype of one class of dsRNA-binding proteins (DRBP). Several human, mouse, Xenopus, Drosophila, viral, and bacterial DRBPs of this class share similar dsRNA-binding motifs (10). The two such motifs present in PKR have been extensively characterized by mutational and structural analyses (11). All dsRNA-binding proteins, however, do not contain these motifs. For example, 2′,5′-oligoadenylate synthetases, another class of interferon-induced enzymes, also require dsRNA for their activation (11). Their dsRNA binding characteristics are quite different from those of PKR and they lack the aforementioned dsRNA-binding motifs (12).

The PKR protein is a dimer (13). Although additional motifs may also contribute, its dimerization is primarily mediated by the same motifs that initiate its dsRNA binding (13, 14). The two functions are, however, independent of each other as shown by genetic and biochemical analyses. We have generated mutants of PKR which have lost the ability of dsRNA binding or dimerization or both (15, 16). These mutants have revealed the importance of PKR dimerization in its biochemical and cellular activities. Because the dsRNA-binding motifs mediate direct protein-protein interaction, different members of the PKR family of DRBPs can heterodimerize. This property of PKR has recently been exploited by us for cloning a new PKR-interacting human protein, PACT (17). As anticipated, PACT is a dsRNA-binding protein as well. But its interaction with PKR is direct and it causes activation and autophosphorylation of PKR in the absence of dsRNA. With the identification of PACT there was reason to expect that there would be other proteins that would interact with both dsRNA and PKR.

We report the cloning and characterization of a human protein that was identified both by its dsRNA binding and by its interaction with PKR. DRBP76 was purified to homogeneity from a HeLa cell extract using its dsRNA-binding property. The same protein was also cloned as a PKR-interactive protein in a yeast two-hybrid screening. DRBP76 is a nuclear protein which can be phosphorylated by PKR in vitro and may contribute to a role for PKR in cell-cycle regulation.

MATERIALS AND METHODS

Protein Purification and N-terminal Microsequencing

HeLa cells were extracted by Dounce homogenization in hypotonic buffer (10 mM HEPES, pH 7.9, 1.5 mM MgCl₂, 2 mM DTT, 1 mM PMSF). Following a short spin to remove cell debris, the lysate was centrifuged...
at 100,000 \times g for 2 h and the resulting ribosomal pellet mixed with hypotonic buffer containing 1 mM KCl. The resuspended material (30 ml) was centrifuged at 100,000 \times g for 2 h and the supernatant/ribosomal salt wash was dialyzed against two changes of 2 liters of 10 mM HEPES buffer, pH 7.9, 20 mM KCl, 1.5 mM MgCl₂, 1 mM DTT, 0.05% Nonidet P-40, 1 mM EDTA, 1 mM PMSF overnight at 4 °C. The dialysate was centrifuged at 15,000 rpm (Jp 20 rotor) for 20 min and the supernatant and pellets were both frozen at −80 °C.

Phosphocellulose Chromatography—Ribosomal salt wash (2 ml) was dialyzed against 500 ml of buffer A (20 mM NaPO₄ buffer, pH 7.0, 20 mM MPP4 (19) and similar to regions of NF90 (20). Clone 9 also encodes a sequence identical to the previously identified incomplete coding region of SH3 domain of PKR. Sequencing of these clones revealed that clone 1 contained sequences encoding the SH3 domain and consequently considered to be candidates for PKR interacting proteins. Sequencing of these clones revealed that clone 1 contained sequences encoding the SH3 domain and consequently considered to be candidates for PKR interacting proteins. The fractions eluted by NaCl gradient were analyzed by Northwestern as described above. The 90- and 110-kDa dsRNA-binding proteins separated.

Electrophoretic Mobility Shift Analysis
Binding of DRBP76 to dsRNA was also assayed by electrophoretic mobility shift assay. 0.1 ng of [γ-32P]ATP-labeled dsRNA probe (22,500 cpm/μl) was incubated with 100 ng of single-stranded DNA, 200 ng of poly(A), and 300 μl of rabbit reticulocyte lysate system (Promega Corp.). 4 μl of binding buffer and 5 μl of purified cellular or purified recombinant DRBP76 were added and incubated an additional 15 min. dsRNA-protein complexes were resolved by separation on 4% non-denaturing polyacrylamide gels and visualized by autoradiography.

In Vitro Interaction of DRBP76 with DNA and PKR
DNase Binding Assay—The interaction of DRBP76 with dsDNA was analyzed by poly(1)-poly(C)-agarose (Amersham Pharmacia Biotech) column equilibrated with the same buffer. The column was washed with the same buffer (15 ml), and then with buffer A containing 0.5 M NaCl and further washed with 0.5 M NaCl in HEPES buffer, pH 7.9 (10 mM HEPES, pH 7.9, 20 mM KCl, 1.5 mM MgCl₂, 1 mM DTT, 0.05% Nonidet P-40, 1 mM EDTA). Finally the protein was eluted from the column with a 0.5–1.0 M NaCl linear gradient (56 ml) in buffer A used to elute at 1 ml/fraction/8-min flow rate. Each of the gradient fractions (15 μl) was run on a 7.5% polyacrylamide gel and Western blot analyses were used to identify the fractions containing dsRNA-binding proteins.

Poly(1)-Poly(C) Chromatography—The fractions containing the 90-kDa dsRNA-binding protein were pooled and dialyzed against 0.2 mM NaCl in buffer B. This was applied to a 1 ml of poly(I)-poly(C)-agarose (Millipore Corp.) for staining with Coomassie Blue. The 90-kDa band was retrieved by reverse transcriptase-PCR using 5′-TTATCTCAGATCAGTACAAGGAAAGTAAATAGCGTC-3′ as forward and 5′-TTGCTGTTGTCAGTAGCCTCCCATAGC-3′ as reverse primers and cDNA reverse transcribed from HeLa S3 cell RNA. A 1.5-kilobase pair XbaI-EcoRI fragment from clone 9, representing the middle of the MPP protein, was ligated into pBlueScript digested with EcoRI and SbaI. The 1-kilobase pair reverse transcriptase fragment was then inserted into this plasmid via the XbaI site and sequenced for correct orientation. This clone which lacks the downstream sequence of MPP4 was designated pBS-MFP4-2BE4. To generate the full-length clone the downstream EcoRI-Xhol 8-kilobase pair fragment of clone 1 was inserted into pET29C via EcoRI and Xhol in combination with the BamHI and EcoRI fragment from pBS-MFP4-2BE4.

Northwestern Assay
Protein were fractionated by SDS-PAGE, transferred to nitrocellulose, and Northwestern analyses were performed as described (21).

Two-hybrid Cloning
 Yeast two-hybrid screening (performed per CLONTECH Laboratories instructions) was used to identify interacting proteins for PKR. Since wild type PKR inhibits yeast cell growth, a mutant PKR was used for this assay. Wild type PKR (L362Q) with only 10% wild type kinase activity was used to construct the interaction of DRBP76 with PKR was analyzed by Northwestern blot analyses using a radiolabeled 85-base pair dsRNA as probe. Two dsRNA-binding proteins were identified by Northwestern blot analysis and the fraction of purified cellular or purified recombinant DRBP76 were added and incubated an additional 15 min. dsRNA-protein complexes were resolved by separation on 4% non-denaturing polyacrylamide gels and visualized by autoradiography.

In Vitro Interaction of DRBP76 with DNA and PKR
DNase Binding Assay—The interaction of DRBP76 with dsDNA was analyzed by poly(1)-poly(C)-agarose (22) of in vitro translated 35S-labeled DRBP76, generated using the TNT T7-coupled reticulocyte lysate system (Promega Corp.). 4 μl of in vitro translation products were diluted with 25 μl of binding buffer (20 mM Tris, pH 7.5, 0.3 mM NaCl, 5 mM MgCl₂, 1 mM DTT, 0.1 mM PMSF, 0.5% Nonidet P-40, 10% glycerol) and incubated with 25 μl of poly(I)-poly(C)-agarose beads at 30 °C for 30 min. The beads were washed 4 times with 500 μl of binding buffer and the bound proteins were analyzed by SDS-PAGE and fluorography. Where indicated the NaCl concentration of binding buffer was changed.

PKR Binding Assay—The interaction of DRBP76 with PKR was analyzed by binding of in vitro translated, labeled DRBP76 with immobilized PKR. The kinase inactive PKR, K286E, and the dsRNA-binding domain of PKR, DRBD, were expressed in bacteria and purified by Ni-agarose affinity chromatography as described (13). 20 μg of purified protein was allowed to bind to 5 μl of packed volume of Ni-agarose beads in the binding buffer (5 mM imidazole, 200 mM NaCl, 20 mM Tris, pH 7.9, 0.5% Nonidet P-40) and then the beads were washed extensively to remove all unbound protein. Four μl of in vitro translated DRBP76 was incubated with 5 μl of beads containing PKR or DRBD or no protein for 30 min at 30 °C in the binding buffer and the specificity of the binding was assessed by including 10 μg of purified PKR or DRBD in the binding buffer during the incubation. After binding, the resin was washed four times with 500 μl of wash buffer (60 mM imidazole, 200 mM NaCl, 20 mM Tris, pH 7.9, 0.5% Nonidet P-40). The bound proteins were then analyzed by SDS-PAGE gels and fluorography as described.

FIG. 1. Purification of DRBPs. A, ribosomal salt wash obtained from HeLa cells (Materials and Methods) was separated by SDS-PAGE, transferred to nitrocellulose, and analyzed by Northwestern blotting using a radiolabeled 80-base pair dsRNA as probe. Two dsRNA-binding proteins of apparent masses of 90 and 110 kDa were detected. B, NaCl gradient fractions from phosphocellulose chromatography were separated by SDS-PAGE and analyzed by Northwestern as described above. The 90- and 110-kDa dsRNA-binding proteins separated. C, fractions 5 and 6 of part B were collected and further purified by poly(I)-poly(C) chromatography. The fractions eluted by NaCl gradient were analyzed by Northwestern. The position of the 90-kDa protein is marked.
In Vitro Kinase Assays

HeLa cells maintained in Dulbecco's modified Eagle's medium containing 10% fetal calf serum were harvested at 70% confluency. The cells were washed with phosphate-buffered saline, pelleted, and lysed in an equal volume of lysis buffer (20 mM Tris, pH 7.5, 5 mM MgCl₂, 50 mM KCl, 400 mM NaCl, 2 mM DTT, 1% Triton X-100, 100 U/ml aprotinin, 0.2 mM PMSE, 20% glycerol). After centrifugation at 10,000 x g for 5 min the supernatants were assayed for PKR activity. PKR was immunoprecipitated from aliquots containing 100 μg of total protein using an anti-PKR monoclonal antibody (Ribogene) in high salt buffer (20 mM Tris, pH 7.5, 50 mM KCl, 400 mM NaCl, 1 mM EDTA, 1 mM DTT, 100 units/ml aprotinin, 0.2 mM PMSE, 20% glycerol, 1% Triton X-100) at 4 °C for 30 min. Protein A-Sepharose (10 μl of slurry) was added for another 30 min. After washing four times with high salt buffer (500 μl) and twice with activity buffer (20 mM Tris, pH 7.5, 50 mM KCl, 2 mM MgCl₂, 2 mM NaCl, 200 units/ml aprotinin, 0.1 mM PMSE, 5% glycerol), the immune complex containing PKR was incubated with activity buffer containing 50 ng of purified eIF-2, 0.1 mM ATP, and 10 mM MgCl₂ with poly(I)-poly(C) as PKR activator. Purified DRBP76 from HeLa cells was added where indicated. Labeled proteins were analyzed by SDS-PAGE and visualized by autoradiography.

Construction of Eukaryotic Expression Vector of DRBP76 with Flag Epitope

To generate a Flag epitope-tagged version of DRBP76 for expression in mammalian cells, the coding region of pDRBP76 was amplified by PCR from p76/PET28C using 5′-GGCCGAGATCTGGAAGACCCAAAATGAGACCAATGCCCCGGATTTTTTG-3′ and 5′-GGCCGGATATGGAAGACCCAAAATGAGACCAATGCCCCGGATTTTTTG-3′ as forward and reverse primers, respectively. The 2.1-kilobase pair PCR product was ligated first into pCR2.1 (Invitrogen) and then the Flag epitope tag was attached to the COOH terminus of p76 by excising the subcloned DRBP76 with KpnI and BglII for insertion in the correct reading frame into a Flag epitope cassette in pBlue-script RS as described previously (23). For mammalian cell expression the Flag epitope-tagged DRBP76 was then excised with KpnI/XhoI digestion and inserted into KpnI/XhoI cut pcDNA3 (Invitrogen).

Immunofluorescence

HT1080 cells on coverslips in 6-well dishes were transfected at 70–80% confluent. Briefly cells in Dulbecco's modified Eagle's medium containing 10% fetal calf serum were transfected using FuGene 6 (Roche Molecular Biochemicals) per the manufacturer's instructions. 1 μg of plasmid DNA per well was used. After 18 h the cells were washed with phosphate-buffered saline, fixed with acetone:methanol (1:1) at room temperature for 2 min, and washed twice with TBST (10 mM Tris, pH 8.0, 150 mM NaCl, 0.02% Tween 20). Coverslips were blocked with 5% non-immune goat serum for 40 min at room temperature. Primary rabbit anti-Flag antibodies (1:1000 in TBST containing 3% bovine serum albumin and 3% non-immune goat serum) were added for 2 h, washed 3 times with TBST and secondary was goat anti-rabbit fluorescein isothiocyanate (1:2000 in TBST plus 3% bovine serum albumin) was added for 30 min. Labeled proteins were analyzed by SDS-PAGE and visualized by autoradiography.

Co-immunoprecipitation

COS 7 cells were transfected with Flag epitope-tagged DRBP76 in pcDNA3 and/or K296R PKR in pRs/CMV (18). Transfected cells were

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**Fig. 2.** Characterization of the purified 90-kDa DRBP. **A,** 100 ng of purified 90-kDa DRBP was separated by SDS-PAGE and detected by silver staining (lane 1) or Northwestern (lane 2). The numbers on the right show the positions of migration of standard proteins of respective molecular masses in kDa. **B,** amino-terminal sequencing of the purified 90-kDa DRBP yielded these amino acids which were identical to the amino termini of MPP4 and NF90.

**Fig. 3.** Sequence of pDRBP76. **A,** the sequence of the protein encoded by the cloned cDNA is shown. The amino acids not present in MPP4 are underlined. Because it encodes a dsRNA-binding protein of calculated molecular mass of 76 kDa, we will call this protein DRBP76. **B,** a schematic representation of the structural features of DRBP76 is shown. **Black boxes** indicate the bi-partite nuclear localization signal, amino acids 398–467 and 524–590. The **hatched box** represents the RG motif, amino acids 640–660. Potential cdc2 phosphorylation sites are marked with a C, while the phosphorylation sites defined by M phase phosphoproteins are marked M.

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**RESULTS**

**Purification of dsRNA-binding Proteins from HeLa Cells—**
The dsRNA-binding proteins were purified from HeLa cell extract. For monitoring the purification, we used Northwestern analysis with a radiolabeled 85-base pair dsRNA (21) as the probe. An excess of unlabeled single-stranded RNA and dsDNA were included in the binding buffer to reduce nonspecific binding of the probe to proteins with affinity for nucleic acids. Two abundant DRBP of apparent molecular mass of 90 and 110 kDa were detected in the extract. Both of these proteins were bound to ribosomes and were solubilized by high-salt extraction (Fig. 1A). The ribosomal salt wash fraction was dialyzed to remove NaCl and the proteins were bound to a phosphocellulose column which was eluted with a NaCl gradient. During this elution, the two DRBPs separated from each other (Fig. 1B). The two proteins shown in lanes 5 and 6 of Fig. 1B were pooled and subjected to further purification by chromatography on poly(I)-poly(C) agarose (Fig. 1C). Fractions shown in lanes 3 and 4 of Fig. 1C were pooled, dialyzed, and concentrated. The preparation was at least 95% pure containing a single protein of an apparent molecular mass of 90 kDa as shown by silver staining (Fig. 2A). The 90-kDa protein was microsequenced and seven residues from its amino terminus were identified (Fig. 2B). The protein sequence matched the amino-terminal sequences of two known human proteins, MPP4 (19) and NF90 (20).

**Cloning of 90-kDa DRBP—** cDNAs encoding the 90-kDa DRBP were also isolated by yeast two-hybrid screening of a HeLa cell cDNA library using a mutant PKR with reduced kinase activity (18) as the bait. Two partially overlapping clones were used to generate a combined clone with the longest open reading frame. The combined cDNAs contained sequence matching that of the previously published partial sequence of MPP4 (19), but extended this at the 3′ end to complete the coding sequence. However, compared with the MPP4 cDNA, it was missing 822 nucleotides at the 5′ end. The missing residues were restored using reverse transcriptase-PCR from HeLa cell RNA as described under “Materials and Methods.” The complete cDNA encodes a protein of calculated molecular mass of 76 kDa (Fig. 3A) that we will call dsRNA-binding protein 76 (DRBP76). It contains 702 residues and its amino-terminal sequence matched perfectly with the sequence of the protein purified from HeLa cells. The sequence matched with the sequence of MPP4, except that it extended further by 92 residues at the COOH terminus (underlined in Fig. 3A). The sequence of DRBP76/MPP4 was also similar, but not identical, to that of NF90 (20).

**Analysis of the primary structure of DRBP76 revealed several interesting features (Fig. 3B). It has a bipartite nuclear localization signal (24), two dsRNA-binding domains that are conserved in other dsRNA-binding proteins including PKR (10) and an RG2 domain (25, 26), a region rich in arginine and glutamine acid residues, that is present in many RNA-binding proteins. In addition, DRBP76 contains five epitopes that are potential sites of phosphorylation in M phase proteins (19) and two epitopes that are present in the substrates of cyclin-dependent protein kinases (27). Several of these structural features were validated by functional analyses of DRBP76 as described below.

**DRBP76 Binds dsRNA—** The mRNA encoded by the cDNA clone of DRBP76 was translated in vitro and yields a protein of an apparent molecular mass of 90 kDa (Fig. 4A, lane 1). Thus, the apparent molecular weight of the recombinant protein was the same as that of the protein purified from HeLa cells. The recombinant protein bound to poly(I)-poly(C) with a high avidity. Salt concentrations as high as 0.5 M NaCl failed to disrupt the dsRNA-protein interaction (Fig. 4A).

The dsRNA binding characteristics of the purified protein from HeLa cells were further analyzed by electrophoretic mobility shift assays. Increasing amounts of DRBP76 shifted increasing amounts of labeled dsRNA (Fig. 4B). The dsRNA-protein complex was heterogeneous probably due to the binding of different numbers of protein molecules to the same dsRNA. As a result, some portion of the complex did not enter the gel (open arrow) whereas the rest formed a broad shifted band (solid arrow). The specificitiy of the binding was confirmed by including in the binding buffer a hundred fold excess of unlabeled poly(I)-poly(C); in the presence of this competitor, the radiolabeled probe failed to bind to the protein and no labeled dsRNA-protein complex was formed (Fig. 4B, lane 7).
DRBP76 Is a Nuclear Protein—To determine the cellular localization, Flag epitope-tagged DRBP76 was transfected into human HT1080 cells and its expression monitored by immunofluorescence analysis using an anti-Flag antibody. DRBP76 was localized exclusively in the nucleus (Fig. 5), consistent with previous studies of MPP4 (19).

DRBP76 Interacts with PKR—The in vitro interaction of DRBP76 and PKR was examined by measuring the binding of radiolabeled DRBP76 synthesized in vitro to PKR immobilized on Ni-agarose. DRBP76 bound specifically to PKR (Fig. 6, lane 3) and, as anticipated, to DRBD (22), the amino-terminal region of PKR that contains a dimerization domain (Fig. 6, lane 4). The specificity of the observed binding was established by performing the binding reaction in the presence of excess soluble PKR (Fig. 6, lane 5) or soluble DRBD (Fig. 6, lane 6). The presence of PKR or DRBD in the solution inhibited the binding of DRBP76 to the affinity resins.

Because DRBP76 interacts with PKR in vitro and it was cloned by its interaction with PKR in yeast, it was clear that the two proteins can bind to each other when they are in close proximity. To determine whether such an interaction occurs in mammalian cells, DRBP76-Flag and an inactive mutant of PKR were co-expressed in COS 7 cells and co-immunoprecipitation assays were performed. The results show that PKR can be co-immunoprecipitated when both are expressed (Fig. 7). However, it appears that only a small fraction of PKR was bound to DRBP76. This could be explained by DRBP76 being a highly abundant cellular protein and consequently the transfected tagged DRBP76 could be competing with the endogenous protein for binding to PKR. Alternatively since only about 20% of PKR is found in the nucleus, most is not available for binding to DRBP, which is a nuclear protein. Thus, it is likely that only a small fraction of the total cellular pools of the two proteins have the opportunity to interact with each other.

DRBP76 Is Phosphorylated by PKR—To test whether DRBP76 is a substrate for PKR we used an in vitro phosphorylation assay. Under the conditions of this assay, PKR phosphorylated itself and eIF-2 efficiently (Fig. 8, lane 1). When purified DRBP76 was added to the assay mixture, it was phosphorylated as well. The observed phosphorylation of DRBP76 was specific because PKR does not phosphorylate several other proteins, such as PACT, TRBP, or DRBP, which also bind to PKR strongly.

**DISCUSSION**

We have previously reported that the same structural motifs of PKR mediate both protein-protein interactions and dsRNA binding (13). Given the independent isolation of DRBP76 as a dsRNA-binding protein and a PKR-interacting protein, it is highly likely that the similar motifs present in DRBP76 also carry out these two independent functions. However, it is possible that the PKR-DRBP76 interaction in yeast was enhanced by dsRNA binding because the two proteins can bind the same dsRNA molecule. Recently a similar protein was isolated using adenovirus VA RNAII as a probe and the apparent molecular
weight and the N-terminal sequence of this protein indicates that it may be identical to DRPB76 (28). The *Xenopus* homolog of DRPB76, MBP4F, was also cloned as a consequence of its dsRNA binding ability and contains the same structural features as DRPB76 (29). Despite the presence of the RG domain which has been shown in other proteins to mediate binding to both single-stranded and double-stranded RNA and DNA (26), MBP4F binds preferentially to dsRNA and RNA-DNA hybrids (29). It will be of interest to determine the role of the RG domain in DRPB76 nucleotide binding.

DRPB76 was cloned by yeast two-hybrid screening as a PKR-interactive protein, a strategy used previously to identify PACT (17). Although both PACT and DRPB76 belong to the same family of DRBPs, their properties are quite distinct. PACT is a cytoplasmic protein which activates PKR whereas DRPB76 is a nuclear protein and is a substrate of PKR. PKR exhibits restricted substrate specificity and does not phosphorylate several PKR-binding proteins including TRBP (30), PACT (17), and the dsRNA-binding domain of PKR (21). Therefore, the phosphorylation of DRPB76 by PKR may be of physiological significance, although this would need to be confirmed in vivo. The nuclear location of DRPB76 suggests it is a substrate for the nuclear pool of PKR, for which thus far no substrates have been identified.

DRPB76 appears to be the full-length version of the M phase-specific phosphoprotein, MPP4 (19). MPP4 was originally cloned as a protein from *M. phase HeLa* cells that was recognized by a monoclonal antibody against phosphorylated epitopes believed unique to M phase proteins. Only a partial cDNA was isolated for MPP4. An antisera generated against MPP4 identified two proteins of apparent size of 90 and 110 kDa from HeLa cells. These are the same sizes as the dsRNA-binding proteins we identified from HeLa cell extracts (Fig. 1A), suggesting that the 110-kDa dsRNA-binding protein is related to DRPB76. MPP4 was shown to be a nuclear protein that becomes hyperphosphorylated during M phase (19).

Another related protein described in the literature is NF-90 (20) although its sequence diverges from that of DRPB76 both in the center of the protein and again at the COOH terminus. Although NF-90 has been claimed to be a transcription factor of NFATs. It does not contain any of the known DNA-binding motifs but, as for MPP4 and DRPB76, it contains the dsRNA-binding motifs.

Recently the DNA-dependent protein kinase was shown to interact with several proteins, one on which had an apparent molecular mass of 90 kDa and the amino-terminal sequence shared by DRPB76 and NF90 (32). While this protein cross-reacted with antiserum against NF90 and could be shown to be a substrate for DNA-protein kinase no direct binding to DNA was detected.

DRPB76 has many interesting structural motifs. It has phosphorylation sites that are hallmarks of M phase proteins (19). These proteins get hyperphosphorylated during M phase by M phase-specific kinases. It also contains sites for phosphorylation by cyclin-dependent kinases (27). The presence of these potential phosphorylation sites suggests that this protein may play a critical role in cell cycle progression. While it is not apparent how dsRNA binding and phosphorylation by PKR may affect this putative function, recent evidence indicates that the activity of PKR is regulated during cell cycle and suggests that PKR may also be involved in cell cycle regulation (9). The other notable features of the protein are its RNA-binding domains. There are two distinct dsRNA-binding domains that also mediate PKR-interaction. In addition, near the COOH terminus there are repeated arginine-glycine motifs which are known to mediate protein-RNA interactions (25, 26). These features suggest that DRPB76 may participate in specific steps of nuclear RNA metabolism. Defining these events and the role of PKR in their regulation should shed light on the nuclear functions of both DRPB76 and PKR.

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