Binding of double-stranded RNA (dsRNA) to PKR induces autophosphorylation and activation. However, the requirement for dsRNA in promoting dimerization and the requirement for dimerization in PKR activation are controversial. We have studied the dsRNA binding and dimerization requirements for the activation of PKR in vivo. Co-expression and immunoprecipitation experiments detected an interaction between the K296P mutant and a bacteriophage T7-epitope-tagged K64E mutant of dsRNA binding domain. In contrast, the K64E/K296P double mutant did not form a detectable dimer with the wild-type dsRNA binding domain. These results support that dimerization of intact PKR with the isolated dsRNA binding domain requires dsRNA binding activity. Expression of the isolated PKR kinase domain (residues 228–551) reduced translation of the reporter mRNA even in the presence of PKR inhibitors. Furthermore, the isolated kinase domain (residues 228–551) undergoes autophosphorylation and sequentially trans-phosphorylates both mutant K296P PKR and wild-type eIF-2α in vitro. In contrast, the isolated kinase domain (residues 264–551) lacking the third basic region was not active. These observations lead us to propose that the dsRNA binding domains on intact PKR inhibit kinase activity and that dsRNA binding to intact PKR induces a conformational change to expose dimerization sites within the dsRNA binding domain thereby promoting dimerization and facilitating trans-phosphorylation and activation.

Cells respond to external stimuli by rapid changes in their translational capacity. Stress, such as growth factor depletion, heat shock, and virus infection, rapidly inhibits protein synthesis through phosphorylation of the α subunit of the eukaryotic translation initiation factor 2 (eIF-2α) (1, 2). Several protein kinases are known to phosphorylate eIF-2α (3). The most ubiquitous eIF-2α kinase in mammalian cells is the double-stranded (ds)1 RNA-activated protein kinase (PKR). PKR expression is induced by interferon as a latent form that is activated upon binding to dsRNA (4). PKR activation and subsequent eIF-2α phosphorylation is the primary mechanism that prevents viral replication as part of the interferon antiviral response (5, 6). Recently, it has become evident that PKR may also play a critical role in regulation of cell growth (7–9), dsRNA-dependent transcriptional regulation (10–13), regulation of differentiation (14, 15), induction of cell apoptosis (16), and suppression of cell transformation (8, 9).

PKR contains two conserved dsRNA binding motifs in its amino terminus and a serine/threonine kinase catalytic domain in its carboxyl terminus (17–19). Extensive mutagenesis studies strongly support that dsRNA binding to PKR is required for dsRNA-dependent activation (19–23). Mutation of the conserved lysine 64 to glutamic acid (K64E) within the first dsRNA binding motif significantly reduces dsRNA binding (19, 24). Although the structural elements for PKR required for dsRNA binding are extensively characterized, little is known about how dsRNA binding leads to PKR activation. Upon binding to dsRNA, PKR becomes autophosphorylated by a mechanism that may require dimerization. The requirement for dimerization is supported by the observations that activation of PKR displays second order kinetics (25), that PKR can be detected as a dimer by size exclusion chromatography (26), and that autophosphorylation of PKR may occur in trans (27). In vitro experiments and in vivo genetic experiments have shown the dsRNA binding domain containing the two dsRNA binding motifs is necessary and sufficient for PKR dimerization (28–30). However, at present, it is unclear whether dsRNA binding promotes dimerization of PKR.

Overexpression of catalytically inactive K296R or K296P (mutants of the invariant lysine within the ATP binding pocket) inhibits the endogenous PKR activity, reduces eIF-2α phosphorylation, increases protein synthesis, and induces cellular transformation (7–9, 31–33). Our previous studies support the hypothesis that the dominant negative phenotype observed by mutant PKR overexpression results from competition for binding to potential dsRNA activators and not from the formation of inactive heterodimers (30).

To further characterize the requirement for dsRNA binding in dimerization and activation of PKR, we studied a double mutant that was defective in both dsRNA binding activity and kinase activity (K64E/K296P). The results show that the ability of the K296P mutant to rescue translation requires dsRNA binding activity. Unexpectedly, co-immunoprecipitation demonstrated that the double mutant K64E/K296P PKR did not dimerize with the intact dsRNA binding domain (1–243). These results suggest that, although dsRNA binding is not required for dimerization of the isolated dsRNA binding domain, dsRNA binding is required for dimerization of the intact PKR molecule. In addition, we demonstrate that the isolated kinase domain (KD) undergoes autophosphorylation and sequentially trans-
phosphorylates K296P and eIF-2a in vitro. The KD also inhibits its protein synthesis in the presence of PKR inhibitors that interfere with dsRNA-dependent activation. These results indicate that the isolated kinase domain (residues 228–551) is a constitutively active kinase independent of the endogenous PKR. Based on these findings, a model is proposed for the mechanism of dsRNA-dependent activation of PKR.

EXPERIMENTAL PROCEDURES

Vector Construction—The expression vectors used in this study contain the same transcription unit utilizing the adenovirus major late promoter and simian virus 40 (SV40) enhancer element. In addition, the vectors contain the SV40 origin for replication in COS-1 cells. The dihydrofolate reductase (DHFR) expression plasmids pD61, pMTVA2, and pMT2 were described previously (34). Briefly, pD61 contains pBR322 as a backbone, whereas pMT2 plasmids contain puC18 as a backbone. pMT2 contains the adenovirus VA1 gene, whereas pMTVA2 does not. The expression plasmid pETFVA2 was described previously (35). The K296P expression vector pETFVA2–K296P, the PKR dsRNA binding domain (BD), amino acid residues 1–243) expression vector pETFVA2–BD, the K64E mutant BD expression vector pETFVA2–K64E–BD, and the PKR kinase domain (amino acid residues 228–551) expression vector pETFVA2–KD were described previously (30). The K64E mutant PKR expression vector was made by digesting pETFVA2–K296P and pETFVA2–K64E–BD with PstI and PvuII. The small fragment from pETFVA2–K64E–BD was isolated and ligated to the large fragment isolated from pETFVA2–K296P to generate pETFVA2–K64E/K296P. The expression vector encoding the third basic region deletion mutant of PKR kinase domain (KDD33, residues 264–551), pETFVA2–KDD33, was made by polymerase chain reaction using the method previously described (30). The sequences of the mutants were determined by the dideoxynucleotide sequencing method (36).

DNA Transfection and Analysis—COS-1 monkey kidney cells were transfected by the DEAE-dextran procedure (37). After 48 h, cells were labeled with Expre35S35S protein labeling mixture (100 Ci/mmol; DuPont NEN) for 20 min in methionine/cysteine-free minimal essential medium (Life Technologies, Inc.). Cell extracts were prepared by lysis in kinase binding buffer (24). For analysis of the dimerization of PKR, equal amounts of DNA (1 µg/ml, unless indicated in the legend) from each vector were co-transfected into COS-1 cells. Cells were labeled and harvested using the same method described above. Proteins were immunoprecipitated using anti-T7-tag monoclonal antibody (Novagen Corp., Madison, WI). Where indicated, the cell extracts were incubated at 30 °C for 30 min in the presence or absence of poly(I):poly(C) (1 µg/ml, Pharmacia Biotech Inc.). The cell extracts and immunoprecipitates were analyzed by SDS-PAGE (38). Gels were fixed in 30% methanol, 10% acetic acid, prepared for fluorography by treatment with En3Hance (DuPont NEN, Boston, MA), and dried. Dried gels were autoradiographed with BIOMAX MR film (Eastman Kodak, Rochester, NY). The co-immunoprecipitated PKR mutants and fragments were quantitated by NIH-Image (Version 1.55b, NIH, Bethesda, MD).

Total RNAs were prepared using the Trizol method according to the procedure provided by the manufacturer (Life Technologies, Inc.) and analyzed by Northern blot hybridization (39) following electrophoresis on formaldehyde-formamide denaturing agarose gels (40). Probes (DHFR and b-actin) were prepared by (α-32P)IdCTP labeling using Rediprimer DNA labeling system (Amersham).

Affinity Binding of PKR Mutants to Poly(I):Poly(C) Agarose—The cell extracts prepared from the transfected COS-1 cells were incubated with poly(I):poly(C) agarose beads (Pharmacia Biotech Inc.) at 30 °C for 30 min in kinase binding buffer (24). The beads were then washed extensively with the same buffer three times at room temperature. The bound proteins were analyzed by SDS-PAGE as described above.

In Vitro Kinase Assay—The K296P, KD–T7, KDD3–T7, eIF-2a, and eIF-2a-51A expression vectors were transfected into COS-1 cells as described above. At 48 h post-transfection, the cells were lysed in kinase binding buffer (24). The expressed proteins were immunoprecipitated using anti-T7-tag antibody and anti-eIF-2a antibody. For trans-phosphorylation assay, the proteins were first mixed as indicated and then immunoprecipitated using the antibodies described. The immunoprecipitated proteins were washed twice with kinase binding buffer (24) and once with kinase assay buffer (20 mM HEPES, pH 7.5, 50 mM KCl, 1.5 mM dithiothreitol, 2 mM Mg(OAc)2, 2 mM MnCl2, and 0.1 mM ATP) and assayed for in vitro kinase activity. The in vitro kinase assay was performed in 25 µl of kinase assay buffer with 5 µM of [γ-32P]ATP at 30 °C for 30 min. The samples were analyzed by SDS-PAGE (38) followed by autoradiography with BIOMAX MR film (Eastman Kodak).

Western Blot Analysis of the Overexpressed PKR Mutants and eIF-2a—The steady-state levels of the overexpressed PKR mutants and eIF-2a were measured in cell extracts obtained as described above. Equal volumes of cell extracts were resolved by SDS-PAGE and electroblotted to nitrocellulose membranes. The membranes were immuno-

RESULTS

The transient transfection system used in this study has been described (30, 34, 35, 41, 42). By using this system, we have previously shown that K296P mutant PKR, the intact dsRNA binding domain (BD, residues 1–243), and the first dsRNA binding motif (D1, residues 1–123) can inhibit endoge-

nous PKR activity and stimulate DHFR translation in this system. To further evaluate the dsRNA binding requirement for mutant PKR to mediate this dominant negative effect, an expression plasmid for synthesis of the PKR double mutant K64E/K296P was constructed. This mutant produces a catalytically inactive PKR that is defective in dsRNA binding capability (19, 24, and see below). The ability for this double mutant to inhibit endogenous PKR activity was compared with K296P, BD, and mutant K64E–BD by co-transfection of COS-1 cells with pD61. DHFR protein synthesis was quantitated by [35S]methionine/cysteine labeling of cells and analyses of har-

vested cell extracts by SDS-PAGE. In parallel, cells were har-

vested for quantitation of mRNA by Northern blot hybridiza-

tion analysis. Transfection of pD61 with the pETFVA2 vector alone into COS-1 cells detected a low level of DHFR synthesis (Fig. 1A, lane 2) that was above the background observed in cells that did not receive pD61 DNA (Fig. 1A, lane 1). Co-transfection of pD61 with either K296P or BD increased DHFR synthesis compared to co-transfection with the pETFVA2 vector alone (Fig. 1A, lanes 2, 3, 5), whereas co-transfection of either K64E/K296P or K64E–BD did not alter DHFR synthesis (Fig. 1A, lanes 4 and 6). Northern blot analysis demonstrated the DHFR mRNA level did not change upon co-transfection with the PKR mutants and fragments (Fig. 1B, lanes 2–6), demonstrating that the changes in DHFR synthesis were due to changes in mRNA translational efficiency.

The dsRNA binding potential for the different PKR mutants and fragments was measured and correlated to translational stimulation. [35S]methionine/cysteine-labeled cell extracts were prepared from the transfected COS-1 cells and were incubated with poly(I):poly(C) agarose beads. After extensive washing, the bound proteins were analyzed by SDS-PAGE. Proteins of 69 kDa and 33 kDa, representing intact PKR and BD of PKR, were observed (Fig. 1C, lanes 3–6). The intensities of the bands corresponding to the K64E mutants were reduced compared to the wild-type K296P and BD, indicating a defect in dsRNA binding (Fig. 1C, lanes 4 and 6 versus 3 and 5). These results support the hypothesis that dsRNA binding activity is required for the dominant negative phenotype as measured by this in vivo translational stimulation assay.

We then asked whether dsRNA binding is required for dimerization of intact PKR. We have previously described an immunoprecipitation assay to detect PKR dimerization that relied on one of the interacting partners being expressed with a bacteriophage T7-epitope tag at the carboxyl terminus (30). K296P PKR or the double mutant K64E/K296P PKR was co-

transfected with K64E–BD–T7 or BD–T7. Analysis of the total cell extracts indicated that K296P, K64E/K296P, BD–T7, and K64E–BD–T7 were expressed in COS-1 cells at a high level (Fig. 2, lanes 1–4). Immunoprecipitation of the cell extracts with limiting amounts of anti-T7 antibody specifically adsorbed each T7-tagged fragment (Fig. 2, lanes 5, 6, 9, and 10) that were not detected in the absence of the T7-epitope tag on the BD (Fig. 2, 1292
Mechanism of dsRNA-dependent PKR Activation

lanes 7, 8, 11, and 12). Immunoprecipitation of T7-tagged K64E/BD detected the expected polypeptide at 33 kDa as well as some co-immunoprecipitation of a 69-kDa polypeptide that represented K296PPKR (Fig. 2, lanes 5 and 9). In addition, the amount of co-immunoprecipitation of K296P PKR with K64E/BD-T7 was increased by incubating the cell extracts with poly(I):poly(C) prior to immunoprecipitation as described under “Experimental Procedures.” T7-epitope-tagged proteins were immunoprecipitated using limiting amounts of anti-T7 monoclonal antibody, and the immunoprecipitates were analyzed by SDS-PAGE followed by autoradiography (lanes 5–12). Under these conditions, approximately 20% of the expressed BD was immunoprecipitated.

Previous results demonstrated that the isolated kinase domain (KD) can phosphorylate eIF-2α and inhibit translation in vivo (30, 33). However, it is not known if the KD directly phosphorylates eIF-2α or whether eIF-2α phosphorylation oc-

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**Fig. 1.** Rescue of DHFR translation by PKR requires dsRNA binding activity. COS-1 cells were co-transfected with the DHFR expression vector pD61 in the presence of the pETFVA expression vector alone (lane 2) or of pETFVA containing the indicated PKR inserts. Lane 1 received only pETFVA DNA. Equal amounts (4 µg/10-cm plate) of the two plasmid DNAs were used. The transfected cells were labeled with [35S]methionine/cysteine and analyzed as described under “Experimental Procedures.” A, analysis of total cell extracts. The migration of DHFR, intact PKR, and the dsRNA binding domain BD are indicated. B, total RNA from cells transfected in parallel was isolated, and the mRNA levels for DHFR and β-actin were analyzed by Northern blot hybridization. C, the expressed PKR mutants were affinity-purified with poly(I):poly(C) agarose beads and analyzed by SDS-PAGE.
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**FIG. 3.** Adenovirus VAI RNA and vaccinia virus E3L do not inhibit isolated intact KD activity. The DHFR reporter gene expression vectors that contain (pMT2) or do not contain (pMTVA) the adenovirus VAI RNA gene were transfected in the absence (lanes 2 and 3) or presence of the wild-type (KD) or mutant (K296P-KD) kinase domain expression vectors (1 μg/ml each DNA; lanes 4–7) as indicated. In lanes 8 and 9, COS-1 cells were co-transfected with the DHFR vector pMTVA (1 μg/ml), the KD vector (0.5 μg/ml), and either the control vector pETFVA (0.5 μg/ml) or the E3L expression vector (0.5 μg/ml) as indicated. The transfected cells were labeled with [35S]methionine/cysteine and lysed with Nonidet P-40 lysis buffer as described under "Experimental Procedures." The total cell extracts were analyzed on SDS-PAGE followed by autoradiography.

The DHFR expression vector that either lacks (pMTVA) or contains (pMT2) the adenovirus VAI RNA gene. DHFR was highly expressed in cells transfected with either pMTVA or pMT2 (Fig. 3, lanes 2 and 3 versus 1). Co-transfection of the wild-type KD with the DHFR reporter plasmid significantly reduced DHFR synthesis (Fig. 3, lanes 2 and 3 versus 4 and 6) even in the presence of VAI RNA provided by pMT2 (Fig. 3, lane 2 versus 4), whereas co-transfection of the K296P-KD with the reporter gene did not alter DHFR synthesis (Fig. 3, lanes 2 and 3 versus 5 and 7). Co-transfection experiments with an expression vector encoding the vaccinia virus E3L gene product, a double-stranded RNA-binding protein that inhibits PKR activity in vivo [pETFVA-E3L, Ref. 23], did not rescue DHFR translation in the presence of KD, even though E3L was expressed at a high level sufficient to inhibit endogenous PKR activity (Fig. 3, lane 9, arrow; Ref. 35). These results support the hypothesis that the isolated kinase catalytic domain of PKR is constitutively active and can directly phosphorylate eIF-2α, independent of COS-1 cell endogenous wild-type PKR.

To further characterize the kinase activity of the isolated kinase domain of PKR, an in vitro kinase assay was performed. Two forms of KD (KD and KDAΔ3) were analyzed that differed in the presence of the D3 domain (residues 226–263 present in KD and absent in KDAΔ3). The T7-epitope-tagged K296P, KD, KDAΔ3, eIF-2α, and eIF-2αs1A were individually expressed in COS-1 cells. The steady-state level of the expressed proteins, analyzed by Western analysis, demonstrated that all proteins were expressed at significant levels (Fig. 4B). The expressed PKR mutants were immunoprecipitated using anti-T7-tag antibody and incubated with [γ-32P]ATP in in vitro kinase assay buffer for activity assay. Under these conditions, KD was autophosphorylated (Fig. 4A, lane 2), whereas K296P and KDAΔ3 was not phosphorylated (Fig. 4A, lanes 1 and 3). The ability of KD to trans-phosphorylate PKR and eIF-2α was then examined using the same in vitro kinase assay. The KD was mixed with K296P, KDAΔ3, eIF-2α, or eIF-2αs1A and then incubated with [γ-32P]ATP in the kinase assay buffer. KD was autophosphorylated in all the samples (Fig. 4A, lanes 4 and 7). In the same experiment, KDAΔ3 and eIF-2αs1A (a Ser → Ala mutation at the site of phosphorylation) were not phosphorylated (Fig. 4A, lanes 5 and 7). The results demonstrate that the isolated kinase domain of PKR (KD) is an active kinase that can phosphorylate substrates similar to wild-type PKR. The results also suggest that the D3 basic region of PKR may contain the in vitro KD-catalyzed phosphorylation site(s).

**DISCUSSION**

The requirements for dsRNA binding to and sequentially activating PKR have been studied intensively through mutagenesis of the PKR dsRNA binding domain as well as dsRNA molecules, such as adenovirus VAI RNA (19–24). The two dsRNA binding motifs in PKR appear to cooperate to promote high affinity stable binding to dsRNA. An intriguing aspect about the dsRNA activation of PKR is that it displays a bell-shaped activation curve, where high concentrations of dsRNA inhibit PKR activation. Based on these observations, two models for the dsRNA-dependent activation of PKR were proposed. One model proposes that PKR functions as a monomer, and activation involves intramolecular autophosphorylation that is...
dependent upon proper occupancy of the two dsRNA binding sites (43). Another model proposes that each PKR molecule binds to one dsRNA molecule, and activation results from bridging of two PKR molecules to elicit intermolecular autophosphorylation (25). This latter model is supported by the kinetics of activation being second order with respect to enzyme concentration (25) and the fact that autophosphorylation can occur in trans (27). In addition to the above models, a third model proposes that PKR exists as a dimer. The presence of dsRNA or other activators of PKR (such as heparin) can stabilize the dimer and promote a conformational change to an active state (44). The support for an RNA-mediated dimerization requirement for PKR activation came from in vitro studies showing that deletion of the two dsRNA binding motifs (amino acids 98–243 deleted) yielded an inactive kinase (45). We have previously characterized a kinase domain fragment containing 15 extra amino acids at the amino terminus of the kinase domain. This enzyme did not detectably dimerize (30); however, it was active in vivo as measured by its ability to increase eIF-2α phosphorylation (30) and to inhibit translation (Fig. 3), consistent with another report (46). In addition, this kinase domain was also active in an in vitro kinase assay. It undergoes autophosphorylation and sequentially trans-phosphorylates the K296P mutant of PKR as well as eIF-2α (Fig. 4). A Ser → Ala mutant at residue 51 in eIF-2α was not phosphorylated (Fig. 4), supporting that phosphorylation of eIF-2α occurred at the natural site.

The additional residues apparently required for kinase activity of the isolated kinase domain belong to a third basic amino acid-rich region (amino acids 233–271) (45). This region is also required for the KD to act as a substrate for the isolated KD. At present it is not known if this region contains a necessary contact point required for intermolecular autophosphorylation or whether it contains the primary phosphorylation sites. Recently, this region was identified to contain phosphorylated threonine residues. The translational inhibition mediated by the kinase domain was not rescued by the PKR inhibitors adenovirus VAI RNA or the vaccinia virus E3L gene product. Both of these PKR inhibitors are known to inhibit PKR activation in a dsRNA-dependent manner through different mechanisms (5, 35). Vaccinia virus E3L is a dsRNA-binding protein that likely binds and sequesters dsRNA PKR activators in the cell (35). In contrast, adenovirus VAI RNA binds to the dsRNA binding site within PKR and prevents its activation by dsRNA (5). Since both of these inhibitors do not inhibit the translational suppression mediated by KD, it suggests that the isolated kinase domain directly phosphorylates eIF-2α, and this does not occur indirectly through phosphorylation of endogenous PKR. We know the endogenous PKR activity is inhibited under conditions of VAI RNA or E3L expression in COS-1 cells (5, 35). Thus, our results suggest that the isolated kinase domain of PKR is a dsRNA-independent and constitutively active eIF-2α kinase. None of the above proposed models for dsRNA-dependent activation can easily explain the observation that the isolated kinase domain of PKR is constitutively active and can phosphorylate eIF-2α in the absence of dsRNA binding and/or dimerization. Thus, there is a need for alternative hypotheses. We propose a model for the dsRNA-dependent activation of PKR to help direct further experimentation to elucidate the possible molecular interactions between the dsRNA binding domains and the kinase domain.

In this study, an in vivo COS-1 cell transfection system was used to study how dsRNA binding and/or dimerization leads to activation of PKR. Transfection of expression vectors encoding either the K296P mutant or the dsRNA binding domain of PKR rescued protein synthesis inhibition of a reporter mRNA that was mediated by endogenous PKR activation (Fig. 1A). However, the ability to rescue protein synthesis was destroyed by mutation of the K64E within the dsRNA binding domain (Fig. 1A). Analysis of poly(I):poly(C) binding affinity showed that the K64E mutation significantly reduced the poly(I):poly(C) binding (Fig. 1C). Thus, the ability to rescue protein synthesis by either the K296P mutant intact PKR or the isolated dsRNA binding domain required dsRNA binding activity, as previously shown (30). The ability of these mutants to dimerize was measured by their ability to co-immunoprecipitate with a T7-bacteriophage-tagged fragment of the dsRNA binding domain (amino acids 1–243). The results demonstrated that mutation of K64E within intact PKR destroyed its ability to dimerize with the isolated dsRNA binding domain (Fig. 2), indicating that dsRNA binding activity was required for dimerization. Whereas addition of poly(I):poly(C) increased dimerization of intact K296P PKR with the K64E-mutated dsRNA binding domain (K64E/BD), poly(I):poly(C) addition had no effect on the inability for the K64E/K296P double mutant to dimerize with the wild-type dsRNA binding domain (Fig. 2). These results are consistent with observations showing a dsRNA-dependent interaction between the dsRNA binding domain and intact PKR (29). However, these findings conflict with mutagenesis studies of the isolated dsRNA binding domain where either K60A (28) or K64E (30), both mutations that destroy dsRNA binding activity, did not prevent dimerization of the mutant dsRNA binding domain with either intact K296P PKR or the isolated dsRNA binding domain. The discrepancy in the results can be explained if dsRNA binding is only required for intact PKR dimerization, but is not required for dimerization of the isolated dsRNA binding domain. We propose that dimerization of the isolated PKR dsRNA binding domain is dsRNA-independent since its dimerization sites are exposed as a consequence of deletion of the kinase domain. This interpretation underscores the caution that needs to be taken in drawing conclusions from mutagenesis experiments using isolated domains of a protein, a situation where the isolated domain may behave differently than when in context of the intact protein.

The above observations lead us to propose a model for the dsRNA-dependent activation of PKR that is consistent with

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previous observations (28–30) (Fig. 5). We propose that the dsRNA binding domains on intact PKR serve as an inhibitor of the kinase, possibly by preventing dimerization or by serving as a pseudosubstrate. Upon dsRNA binding to intact PKR, a conformational change occurs that exposes the site(s) for dimerization. At the same time, the conformational change liberates the kinase domain from the inhibitory dsRNA binding domain and promotes autophosphorylation in trans. In contrast to intact PKR, the isolated dsRNA binding domain BD can dimerize independent of dsRNA, and the isolated kinase domain can phosphorylate eIF-2α without dimerization.

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