The endoplasmic reticulum (ER)-resident protein kinase PERK attenuates protein synthesis in response to ER stress through the phosphorylation of translation initiation factor eIF2α at serine 51. ER stress induces PERK autophosphorylation at several serine/threonine residues, a process that is required for kinase activation and phosphorylation of eIF2α. Herein, we demonstrate that PERK also possesses tyrosine kinase activity. Specifically, we show that PERK is capable of autophosphorylating on tyrosine residues in vitro and in vivo. We further show that tyrosine 615, which is embedded in a highly conserved region of the kinase domain of PERK, is essential for autocatalytic activity. That is, mutation of Tyr-615 to phenylalanine compromises the autophosphorylation capacity of PERK and the phosphorylation of eIF2α in vitro and in vivo. The Y615F mutation also impairs the ability of PERK to induce translation of ATF4. Immunoblot analyses with a phosphospecific antibody confirm the phosphorylation of PERK at Tyr-615 both in vitro and in vivo. Thus, our data classify PERK as a dual specificity kinase whose regulation by tyrosine phosphorylation contributes to its optimal activation in response to ER stress.

Control of gene expression at the translational level plays critical roles in cell growth, proliferation, and tumor development (1–3). A big part of the translational regulation is exerted at the level of initiation when the ribosome is recruited to an mRNA and positioned at the initiation codon (4). A critical event in this process is the phosphorylation of the α subunit of translation initiation factor eIF2 at serine 51, a modification that blocks initiation (5). This is because phosphorylated eIF2 acts as a dominant inhibitor of the guanine exchange factor eIF2B and prevents recycling of eIF2 between successive rounds of protein synthesis (6). Phosphorylation of eIF2α is mediated by kinases that respond to distinct forms of stress (7).

The eIF2α kinase family includes the heme-regulated inhibitor, whose activity is prevented by heme in vitro and in vivo, and which becomes activated when cells are deficient in iron or heme or exposed to oxidative stress (8, 9). The general control non-derepressible-2 (GCN2) is activated by uncharged tRNA as a result of amino acid starvation resulting in the induction of amino acid biosynthetic genes (10, 11). The activity of the endoplasmic reticulum (ER) resident protein kinase PERK/PEK is induced by the presence of unfolded proteins in the ER and results in a decrease of protein synthesis to prevent the accumulation of improperly folded proteins (12, 13). Finally, the interferon-inducible protein kinase PKR, the prototype of the eIF2α kinases, is activated by double-stranded RNA produced during virus replication and results in the inhibition of viral and host protein synthesis (14, 15). Each of these enzymes exhibit a number of significant sequence similarities between them, particularly in the protein kinase domain, which explains their specificity for eIF2α.

Activation of eIF2α kinases requires their autophosphorylation on several serine and threonine residues (5). Recent findings, however, provided strong evidence for the regulation of eIF2α kinase activity by tyrosine phosphorylation. Specifically, we demonstrated that tyrosine phosphorylation is required for optimal activation of the eIF2α kinase PKR in response to double-stranded RNA or virus infection (16). Furthermore, we demonstrated that site-specific tyrosine phosphorylation of PKR is an important link between interferon signaling and translational control (17). Prompted by our findings with PKR, we have investigated whether other eIF2α kinases are similarly controlled by tyrosine phosphorylation. Herein, we demonstrate the dual specificity of PERK and its essential role in optimal activation of the kinase and eIF2α phosphorylation in response to ER stress.

EXPERIMENTAL PROCEDURES

Cell Cultures and Treatments—The human A549 cells were maintained in F12K medium (Cellgro), whereas monkey kidney COS-1 cells and isogenic PERK−/− and PERK+/− MEFs (18) were maintained in Dulbecco’s modification of Eagle’s medium. Both media were supplemented with 10% fetal bovine serum.
heat-inactivated fetal bovine serum (Invitrogen) and antibiotics (penicillin/streptomycin, 100 units/ml). TG (Sigma) was used at a final concentration of 1 μM, whereas infection with VSV (Indiana strain) was performed at multiplicity of infection 10 as described (19).

**Immunoprecipitation and Immunoblot Analyses**—In general, the immunoprecipitations and immunoblots were performed as described (20). For detection of tyrosine phosphorylation of endogenous PERK, cells (~1 × 10^7) seeded onto 150-mm plates were washed with ice-cold phosphate-buffered saline (140 mM NaCl, 15 mM KH_2PO_4, pH 7.2, 2.7 mM KCl), and proteins were extracted with 600 μl of lysis buffer containing 130 mM Tris-HCl, pH 7.4, 2% SDS, and 1% β-mercaptoethanol. After incubation on ice for 20 min, the lysates were sonicated in a 550 Sonic Dismembrator (10% output, twice with 10-s pulses on ice) and cleared by centrifugation at 14,000 × g for 10 min at 4 °C. The supernatant was transferred to a fresh tube and heated at 100 °C for 10 min. Five hundred μl of protein extracts were diluted to a final volume of 2.5 ml in ice-cold phosphate-buffered saline. Approximately 10 μg of antibody and 100 μl of packed volume of protein G-agarose were used for immunoprecipitation of each sample. The samples were incubated with the antibody and Sepharose beads overnight under rotation at 4 °C. The supernatant was transferred to a fresh tube and washed 3 times with ice-cold phosphate-buffered saline before being subjected to SDS-PAGE. Proteins were transferred and washed 3 times with ice-cold phosphate-buffered saline (140 mM NaCl, 15 mM KH_2PO_4, pH 7.2, 2.7 mM KCl), and proteins were extracted with 600 μl of lysis buffer containing 130 mM Tris-HCl, pH 7.4, 2% SDS, and 1% β-mercaptoethanol.

First we examined the tyrosine phosphorylation of endogenous PERK. We employed human A549 cells, which contain high levels of PERK among many cell lines tested (data not shown), to detect its tyrosine phosphorylation. Due to rapid dephosphorylation and degradation of tyrosine-phosphorylated PERK in buffers that preserve its activity (data not shown), we attempted the immunoprecipitation of endogenous PERK from protein extracts prepared under denaturing condition. We found that immunoprecipitated PERK cross-reacted with the presence of basal levels of tyrosine-phosphorylated kinase in proliferating cells. The intensity of the signal with the anti-phosphotyrosine antibody was more highly induced after treatment with TG indicating that increased tyrosine phosphorylation of PERK coincides with its activation in response to ER stress (Fig. 1A, panel a, lanes 2 and 3). Immunoblot analysis with anti-PERK specific antibodies showed that tyrosine-phosphorylated PERK co-migrated with the immunoprecipitated kinase (panel b). Furthermore, immunoblot analysis of the denatured protein extracts before and after immunoprecipitation with anti-PERK antibodies showed that a significant amount of endogenous PERK was immunodepleted under the conditions of the experiments (Fig. 1A, panel c). To further verify the tyrosine phosphorylation of endogenous PERK, protein extracts prepared under the same denaturing conditions were immunoprecipitated with anti-phosphotyrosine-specific antibody followed by immunoblotting with anti-PERK antibodies (Fig. 1B, panel a). We detected PERK in untreated cells (Fig. 1B, panel a, lane 1), consistent with the presence of basal levels of tyrosine phosphorylation of the kinase. Interestingly, treatment with TG resulted in the immunoprecipitation of two forms of PERK that differed in size (panel a, lanes 2 and 3); the slower (upper) migrating form of PERK was indicative of its hyperphosphorylation in response to ER stress as documented in many studies (see also below). Immunoblot analysis of the denatured protein extracts before and after immunoprecipitation for PERK indicated that a sig-
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![Image of autoradiograph and schematic diagram]

**FIGURE 1.** Tyrosine phosphorylation of PERK in vivo. A, A549 cells were left untreated or treated with 1 μM TG for the indicated time points. Denatured protein extracts (see “Experimental Procedures”) were subjected to immunoprecipitation with anti-PERK rabbit polyclonal antibodies followed by Western blot (WB) analysis first with anti-phosphotyrosine PY99 antibody (anti-Tyr(P) pY Ab; panel a) and second with anti-PERK antibodies (panel b). Immunoprecipitation of protein extracts from cells treated with TG for 2 h with rabbit IgG was used as specificity control (Immunoprecipitation of protein extracts from cells treated with TG for 2 h). Note that the tyrosine-phosphorylated PERK (panel a) coincides with the slow migrating hyperphosphorylated form of PERK (panel b). Equal amounts of protein extracts before and after the immunoprecipitation (IP) with anti-PERK antibodies were subjected to immunoblot analysis with anti-PERK antibodies to assess the immunodepletion of PERK (panel c). B, denatured protein extracts from A549 cells untreated or treated with 1 μM TG for the indicated times were employed for immunoprecipitation with anti-phosphotyrosine antibody (Tyr(P)-99 monoclonal antibody (anti-pY Ab)). The immunoprecipitates were subjected to Western blot analysis with anti-PERK antibodies (panel a). The slower migrating bands (panel a, lanes 2 and 3) represent the hyperphosphorylated form of PERK. Equal amounts of protein extracts before and after the immunoprecipitation with anti-phosphotyrosine antibody were subjected to Western blot analysis with anti-PERK antibodies (panel b). The slower migrating bands (panel b, lanes 2 and 3) represent the hyperphosphorylated form of PERK. A and B, whole cell extracts (50 μg of protein) from PERK mice (lanes 1 and 2) and PERK WT (lanes 3 and 4) were used as controls for anti-PERK antibody. The data represent one of two reproducible experiments.

 significent portion of the kinase was immunodepleted after incubation with the anti-phosphotyrosine antibody (Fig. 1B, panel b, compare lanes 4–6 with lanes 1–3). In fact, a higher amount of PERK was immunodepleted with the anti-phosphotyrosine antibody after ER stress (Fig. 1B, panel b, lanes 5 and 6) consistent with the increased detection of tyrosine-phosphorylated PERK by immunoprecipitation (panel a, lanes 2 and 3). These data supported the tyrosine phosphorylation of endogenous PERK.

Next, we tested the tyrosine phosphorylation of recombinant PERK. Because GST-PERK WT expressed in bacteria is already autophosphorylated, recombinant GST-PERK WT was first treated with calf intestinal phosphatase to remove the pre-existing autophosphorylation and then subjected to autokinase assay in the presence of [γ-^32P]ATP. Phosphoamino acid analysis detected the autophosphorylation of the kinase mainly on serine and threonine residues but also on tyrosine residues (Fig. 2A). To verify tyrosine phosphorylation, GST-PERK WT was treated with a recombinant GST fusion protein consisting of the tyrosine-specific phosphatase TC-PTP (29) (Fig. 2B). Treatment with the phosphatase rendered tyrosine phosphorylation of GST-PERK WT undetectable (panel a, lane 1) as opposed to treatment with the catalytically inactive phosphatase mutant GST-TC-PTP(D182A), which did not interfere with the detection of the tyrosine-phosphorylated kinase (panel a, lane 3). The noted differences in migration between the active and inactive GST-PTP proteins (Fig. 2B, panel b) were due to hyperphosphorylation and retardation of the active kinase in polyacrylamide gels as reported (19). Given that detection of GST-PERK-K618A with anti-phosphotyrosine antibodies was not possible (panel a, lanes 2 and 4), we conclude that PERK does not possess an epitope that mimics tyrosine phosphorylation.

Our previous work identified Tyr-293 of PKR as a functional autophosphorylation site (16). Tyr-293 lies within the catalytic subdomain II of PKR and is highly conserved among all homologs of the eIF2α kinases (16) (Fig. 3A). Given the essential role of Tyr-293 in PKR activation (16), we were interested to examine whether this invariant Tyr-615 is phosphorylated in PERK and whether this modification affects PERK activity. We first tested whether the phosphospecific anti-PKR-Tyr(P)-293 antibody is suitable for detection of phosphorylated PERK at Tyr-615 given the high homology between the two kinases in...
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FIGURE 3. Tyrosine 615 of mouse PERK is essential for kinase activity in vitro. A, sequence alignment of the region that corresponds to Tyr-293 of human (h) PKR in human PERK, GCN2 (general control non-derepressible-2), and heme-regulated inhibitor. The positions of the conserved Tyr and Lys residues are highlighted in red and orange, respectively. B, detection of PERK phosphorylation at Tyr-615 in vitro. Purified GST fusion proteins (10 ng) consisting of either PERK WT or the indicated mutants of PERK were subjected to immunoblotting with a phosphospecific antibody against Tyr-293 of human PKR (panel a) in the presence of non-phosphorylated PERK peptide (KYDCCNYAIRIR; lanes 1 – 3) or PERK peptide phosphorylated at Tyr-615 (KYDCC-NyAIKRIR, pY7) as phosphorytosine; lanes 4 – 6). Total levels of the GST-PERK proteins were detected by immunoblotting with anti-GST antibodies (panel b). The data represent one of two reproducible experiments. C and D, kinase activity of the GST-PERK-Y615F mutant. C, 10 ng of purified GST-PERK WT or GST-PERK-Y615F was used for immunoblotting with anti-phosphotyrosine 4G10 monoclonal antibody (panel a) followed by immunoblotting with anti-GST antibodies (panel b). D, 10 ng of the indicated GST-PERK proteins were used for autophosphorylation in the presence of 10 ng of purified histidine-tagged eIF2α and [γ-32P]ATP. Half of the reactions were subjected to SDS-PAGE and autoradiography to detect phosphorylated GST-PERK (panel a) or eIF2α (panel b), whereas the rest was subjected to immunoblotting with anti-GST antibodies and detection with enhanced chemiluminescence (1-min exposure) to detect the levels of the GST-PERK proteins (panel c). C and D, the data represent one of three reproducible experiments.

this region (Fig. 3A). To verify the specificity of the antibody for PERK, we performed the immunoblotting in the presence of a PERK peptide bearing Tyr-615 in either non-phosphorylated or phosphorylated form. We noticed that, in the presence of the non-phosphorylated PERK peptide, the phosphospecific antibody reacted with catalytic active GST-PERK (Fig. 3B, panel a, lane 1) but not with GST-PERK-K618A (lane 2) or GST-PERK-Y615F (lane 3). Contrary to this, the phosphorylated at Tyr-615 PERK peptide abolished the recognition of active GST-PERK with the phosphospecific anti-PKR-Tyr(P)-293 antibody (panel a, lane 4). These data confirmed the suitability of the anti-PKR-Tyr(P)-293 antibody for the detection of PERK phosphorylated at Y615. When, immunoblotting were performed with antiphosphotyrosine antibody, we noticed that detection of tyrosine phosphorylation of GST-PERK-Y615F was not possible (Fig. 3C, panel a, lane 2). This finding indicated that either Tyr-615 is the only tyrosine phosphorylation site on GST-PERK or Y615F mutation has had a significant effect on GST-PERK autophosphorylation. To distinguish between the two possibilities we tested the overall catalytic activity of GST-PERK-Y615F in terms of autophosphorylation and eIF2α phosphorylation in kinase assays with [γ-32P]ATP (Fig. 3D). We found that autophosphorylation of GST-PERK-Y615F was significantly compromised to undetectable levels as was the case with the catalytic inactive GST-PERK-K618A mutant (panel a, compare lanes 2 and 3). Furthermore, the ability of GST-PERK-Y615F to phosphorylate recombinant eIF2α remained equally undetectable (panel b, lane 3). These findings provided evidence for an essential role of Tyr-615 in PERK activation in vitro.

The above data prompted us to examine the role of Tyr-615 in PERK activation in vivo. To do so we employed Myc-tagged forms of wild type and mutant PERK proteins for transient expression in COS1 cells. Expression of Myc-tagged PERK WT led to the induction of its phosphorylation at Tyr-615 (Fig. 4A, panel a) and phosphorylation of endogenous eIF2α phosphorylation at Ser-51 (panel c) as opposed to Myc-tagged PERK-K618A and PERK-Y615F mutants, which were not phosphorylated at Tyr-615 (panel a) and were unable to increase phosphorylation of endogenous eIF2α (panel c). Also, the overall autophosphorylation capacity of the Myc-tagged PERK-Y615F and PERK-K618A mutants was abolished compared with the autophosphorylation of Myc-tagged PERK WT (Fig. 4A, panel e; compare lanes 3 and 4 with lane 1). The lack of the autocatalytic activities of the PERK mutants was further sup-
ported by their inability to mediate the phosphorylation of recombinant eIF2α (panel f). When the same Myc-tagged forms of PERK were expressed in a separate experiment and probed with anti-phosphotyrosine antibody, we noticed that detection of phosphorylation was possible for Myc-tagged PERK WT only after treatment with TG (Fig. 4B, panel a, lane 6). Contrary to active PERK, detection of tyrosine phosphorylation of the Myc-tagged PERK mutants with the anti-phosphotyrosine antibody was not possible (panel a, lanes 7 and 8). Activation of Myc-tagged PERK WT was also evident by its slow migration in the polyacrylamide gels compared with Myc-tagged PERK mutants (panel b). Furthermore, a higher amount of endogenous eIF2α was phosphorylated at Ser-51 in cells expressing Myc-tagged PERK WT than in cells expressing the Myc-tagged PERK mutants (panel c), consistent with the impaired catalytic activity of the PERK mutants. These data further substantiated an important role for Tyr-615 in PERK activation in vivo.

We next examined Tyr-615 phosphorylation of PERK in vivo. Phosphorylation of Myc-tagged PERK WT at Tyr-615 became evident in transiently transfected COS1 cells after immunoprecipitation with anti-Myc antibody and immunoblotting with the phosphospecific anti-PKR-Tyr(P)-293 antibody (Fig. 5A, panel a). In fact, Tyr-615 phosphorylation of Myc-tagged PERK WT coincided with its phosphorylation at Thr-980 within the activation loop of the kinase (panel b). Contrary to active PERK WT, neither of the Myc-tagged PERK mutants was recognized by the phosphospecific antibody (panels a and b). Detection of Tyr-615 phosphorylation was also possible in whole cell extracts from COS1 cells transiently transfected with Myc-tagged PERK WT but not with Myc-tagged PERK-K618A (Fig. 5B, panel a). In these experiments we noticed a shift of phosphorylated Myc-tagged PERK WT after infection with VSV (panel a, lane 5), which activates PERK (19), or after treatment with TG (panel a, lane 8). These differences in PERK mobility were further verified by immunoblotting with anti-Myc antibody (panel b) and are explained by a higher induction of PERK activity in response to VSV infection or ER stress. This interpretation is also supported by the higher levels of endogenous eIF2α phosphorylation at Ser-51 in response to these treatments (panel c, lanes 5 and 8). To further substantiate Tyr-615 phosphorylation of PERK in vivo, we employed human
A549 cells for immunoprecipitation of the endogenous kinase with the phosphospecific anti-PKR-Tyr(P)-293 antibody from protein extracts prepared under denaturing conditions. Similarly to experiments described in Fig. 1, we detected a fair amount of PERK to be immunoprecipitated with the phosphospecific anti-PKR-Tyr(P)-293 antibody (Fig. 5C, panel a). A fraction of immunoprecipitated PERK displayed a slower mobility after treatment with TG compared with PERK immunoprecipitated from untreated cells (panel a, compare lanes 2 and 3 with lane 1), indicating the hyperphosphorylation of the kinase in response to ER stress. Immunoblot analysis of whole protein extracts before and after immunoprecipitation revealed that a significant fraction of PERK was immunodepleted by the phosphospecific PKR-Tyr(P)-293 antibody (panel b, compare lanes 5 and 6 with lane 4). Collectively, these data showed that Tyr-615 phosphorylation of PERK takes place in vivo and is important for the activation of the elf2α kinase in response to ER stress.

To address the functionality of Tyr-615 phosphorylation, we examined the ability of PERK-Y615F mutant to stimulate translation of ATF4 mRNA, which is solely dependent on elf2α phosphorylation (27). To this end, PERK−/− MEFs were co-transfected with Myc-tagged PERK WT, PERK-K618A, or PERK-Y615F, and a fusion of the luciferase reporter gene was transcribed to an mRNA containing either ATF4 5′-untranslated region (UTR) or an ATF4 5′-UTR mutant lacking ORF1 function (Δ1) (27) (Fig. 5D). We found that neither of the PERK mutants was capable of stimulating the activity of the reporter gene as opposed to PERK WT, which did so efficiently (Fig. 5D). Induction of luciferase activity in cells transfected with ATF4 mutant ORF1 (Δ1)/luciferase and PERK WT was not possible (Fig. 5D), supporting previous data that the ATF4/luciferase reporter assay faithfully represents translation of endogenous ATF4 mRNA as a result of elf2α phosphorylation (27). These data provided a link between tyrosine phosphorylation of PERK and translational control of ATF4 mRNA.

**DISCUSSION**

Our findings support the function of PERK as a dual specificity kinase and are in line with an earlier study showing the tyrosine phosphorylation of recombinant PERK by mass spectrometry (30). Although tyrosine phosphorylation of PERK in vitro is clearly an autophosphorylation event, phosphorylation of PERK tyrosine residues in vivo could also require the activity of other kinase(s) in a manner similar to that previously documented for PKR (17). Experiments with phosphospecific antibodies indicated that untreated cells contained basal levels of tyrosine-phosphorylated endogenous PERK. For example, a significant amount of endogenous PERK was immunoprecipitated with anti-phosphotyrosine antibody (Fig. 1B) or anti-PKR-Tyr(P)-293 antibody (Fig. 5C) from untreated cells. These data suggest that tyrosine phosphorylation of PERK takes place as soon as the protein is produced within the cells. This interpretation is also supported by the finding that ectopically expressed PERK is phosphorylated at Tyr-615 before induction of ER stress (Fig. 5A). Nevertheless, when ectopically expressed PERK was immunoprecipitated before ER stress treatment, tyrosine phosphorylation with anti-phosphotyrosine anti-body was not detected (Fig. 4B, lane 2). The most conceivable interpretation is that anti-PKR-Tyr(P)-293 antibody is more sensitive for detecting phosphorylated PERK than the anti-phosphotyrosine antibody. It is also of interest that immunoprecipitation of endogenous PERK with the anti-phosphotyrosine antibody yielded two distinct forms of the tyrosine-phosphorylated kinase based on its migration in polyacrylamide gels (Fig. 1B, panel a). These forms, however, were not evident when endogenous PERK was immunoprecipitated with the anti-PKR-Tyr(P)-293 antibody (Fig. 5C, panel a). This finding may indicate the presence of two different pools of tyrosine-phosphorylated PERK, one of which is enriched with PERK phosphorylated at Tyr-615. Consistent with this interpretation, immunoprecipitation of endogenous PERK with anti-PERK antibodies followed by immunoblotting with the anti-phosphotyrosine antibody yielded a single band of tyrosine-phosphorylated PERK, which was more highly induced after TG treatment (Fig. 1A, panel a). This result may also imply that the anti-PERK antibodies display a higher affinity for a single pool of tyrosine-phosphorylated kinase during immunoprecipitation for reasons that are not immediately clear. Despite the noted differences in the specificity of the antibodies, when examined in concert these experiments supported the notion that PERK is indeed modified by tyrosine phosphorylation in untreated cells and tyrosine phosphorylation of PERK is more highly induced in response to ER stress.

Although more than one tyrosine residue of PERK is likely to be modified by phosphorylation, our data provide strong evidence that Tyr-615 plays a critical role in kinase activation (Figs. 3 and 4). The Y615F mutation is impaired in both basal and stress-induced (global) PERK phosphorylation. The latter is consistent with the notion that Tyr-615 phosphorylation could be altering the overall structure of the N-terminal lobe of the kinase to affect catalysis. PERK phosphorylation at Tyr-615 is detected before ER stress treatment (Figs. 5, A–C) consistent with the notion that its phosphorylation may prime the elf2α kinase to full-scale activation. How Tyr-615 becomes phosphorylated is not presently known. Conformational changes of full-length PERK caused by the presence and function of the stress-sensing luminal domain (31) could alter the accessibility of Tyr-615 for autophosphorylation and possible phosphorylation by another kinase(s). It is also possible that tyrosine phosphorylation of PERK, PKR, and potentially of other elf2α kinases is a process that takes place during protein synthesis and before acquisition of the final conformation as has been recently suggested for tyrosine-phosphorylated glycogen synthase kinase 3β (32). This would be consistent with the detectable basal levels of tyrosine-phosphorylated kinase in untreated cells. Our previous data with PKR demonstrated that phosphorylation at Tyr-293 is required for optimal phosphorylation of Thr-446, which lies within the activation loop of the kinase (16). Consistent with this, Tyr-615 phosphorylation of PERK coincides with phosphorylation at Thr-980 within the activation loop of the kinase (Fig. 5A), further supporting a positive role of Tyr-615 phosphorylation in PERK activation.

Concerning the biological relevance of our findings, the ability of PERK to act as a dual specificity kinase may be associated with the operation of novel signaling pathways that control ER...
homeostasis and its response to various forms of stress. Structural and functional data for the kinase domain of PKR in bacteria and yeast have led to the conclusion that the only substrate of the kinase is eIF2α (33, 34). However, in mammalian cells tyrosine phosphorylation of the eIF2α kinases may affect substrate specificity leading to phosphorylation of proteins other than eIF2α. Such post-translational modifications could account for the phosphorylation of previously identified substrates of PKR and PERK including the viral protein Tat (35, 36) and the cellular proteins IkBα (37), NFAR-1/2 (38, 39), TC-PTP (28), and p53 (40), all of which lack sequence or structural homology with eIF2α. Thus, identification and characterization of novel substrates based on the properties of eIF2α kinases as dual specificity kinases may be of immense importance for the design of strategies to combat human diseases such as diabetes and obesity as well as cancer.

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