15. Ota, Y. & Samelson, L. E. The product of the proto-oncogene c-*cbl*: a negative regulator of the Syk tyrosine kinase. *Science* **276,** 418–420 (1997).
16. Holm, L. & Sander, C. Dali: a network tool for protein structure comparison. *Trends Biochem. Sci.* **20,** 478–480 (1995).
17. Kretsinger, R. H. EF-hands embrace. *Nature Struct. Biol.* **4,** 514–516 (1997).
18. Essen, L. O., Perisic, O., Cheung, R., Katan, M. & Williams, R. L. Crystal structure of a mammalian phosphoinositide-specific phospholipase Cδ. *Nature* **380,** 595–602 (1996).
19. de Beer, T., Carter, R. E., Lobel-Rice, K. E., Sorkin, A. & Overduin, M. Structure and Asn-Pro-Phe binding pocket of the Eps15 homology domain. *Science* **281,** 1357–1360 (1998).
20. Becker, S., Groner, B. & Muller, C. W. Three-dimensional structure of the Stat3β homodimer bound to DNA. *Nature* **394,** 145–151 (1998).
21. Chen, X. *et al*. Crystal structure of a tyrosine phosphorylated STAT-1 dimer bound to DNA. *Cell* **93,** 827–839 (1998).
22. Hatada, M. H. et al. Molecular basis for the interaction of the protein tyrosine kinase ZAP-70 with the T-cell receptor. *Nature* **377,** 32–38 (1995).
23. Eck, M. J., Shoelson, S. E. & Harrison, S. C. Recognition of a high-affinity phosphotyrosyl peptide by the Src homology-2 domain of $p56^{lck}$. *Nature* **362,** 87–91 (1993).
24. Otwinowski, Z. & Minor, W. Processing of X-ray diffraction data collected in oscillation mode. *Methods Enzymol.* **276,** 307–326 (1997).
25. Collaborative Computational Project Number 4. The CCP4 suite: Programs for protein crystallography. *Acta Crystallogr. D* **50,** 760–776 (1994).
26. Jones, T. A., Zhou, J. Y., Cowan, S. W. & Kjeldgaard, M. Improved methods for building protein models in electron density maps and the location of errors in these models. *Acta Crystallogr. A* **47,** 110–119 (1991).
27. Brunger, A. *X-PLOR Version 3.0: A System for Crystallography and NMR* (Yale University Press, New Haven, 1992).
28. Navaza, J. (ed.) *AMoRe: A New Package for Molecular Replacement* (SERC, Daresbury, UK, 1992).
29. Kraulis, P. J. MOLSCRIPT: a program to produce both detailed and schematic plots of protein structures. *J. Appl. Crystallogr.* **24,** 946-950 (1991).
30. Nicholls, A., Sharp, K. A. & Honig, B. Protein folding and association: insights from the interfacial and thermodynamic properties of hydrocarbons. *Proteins Struct. Funct. Genet.* **11,** 281-296 (1991).

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Correspondence and requests for materials should be addressed to M.J.E. (e-mail: eck@red.dfci.harvard.edu). Atomic coordinates have been deposited with the Protein Data Bank (Brookhaven National Laboratory) under accession numbers 2cbl and 1b47.

erratum

Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase

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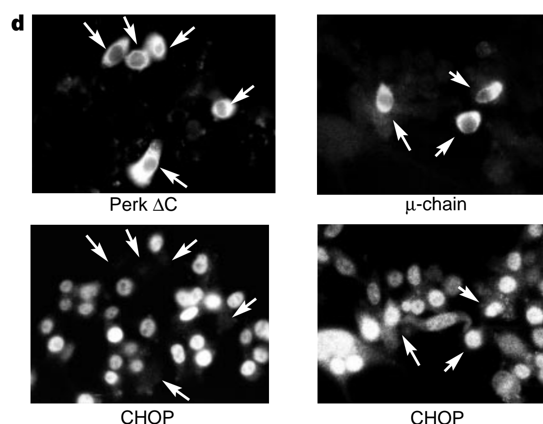
As a result of a production error, the arrows in Fig. 3d were inappropriately aligned. The correct figure is reproduced below.



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Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase

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Protein synthesis and the folding of the newly synthesized proteins into the correct three-dimensional structure are coupled in cellular compartments of the exocytosis pathway by a process that modulates the phosphorylation level of eukaryotic initiation factor-2α (eIF2α) in response to a stress signal from the endoplasmic reticulum (ER)[1,2]. Activation of this process leads to reduced rates of initiation of protein translation during ER stress[3]. Here we describe the cloning of *perk*, a gene encoding a type I transmembrane ER-resident protein. PERK has a lumenal domain that is similar to the ER-stress-sensing lumenal domain of the ER-resident kinase Ire1, and a cytoplasmic portion that contains a protein-kinase domain most similar to that of the known eIF2α kinases, PKR and HRI. ER stress increases PERK's protein-kinase activity and PERK phosphorylates eIF2α on serine residue 51, inhibiting translation of messenger RNA into protein. These properties implicate PERK in a signalling pathway that attenuates protein translation in response to ER stress.

In eukaryotic cells, the folding of proteins that are destined to be secreted or membrane-bound takes place in the ER. This process is impeded when cells are deprived of essential nutrients or energy sources or exposed to toxins that perturb the specialized environment of the ER[4]. The accumulation of incorrectly folded proteins triggers an ER-stress response that has at least two distinct components. The first, known as the unfolded-protein response (UPR), consists of the transcriptional induction of genes that encode ER-resident proteins such as Grp78, Grp94 and protein disulphide isomerase. These proteins are believed to promote the folding of newly synthesized peptides in the ER lumen[5,6]. The second component consists of a profound and rapid repression of protein synthesis[3]. Both responses can be rationalized in terms of the need to relieve ER stress: the first response increases the capacity of the ER to actively fold proteins and the second decreases the demands made on the organelle by attenuating protein-synthesis rates[2].

The mediators of the UPR have been well characterized, first in yeast[7,8] and, more recently, in mammalian cells[9,10]. The sensor of ER stress in both types of cell is an ER-resident transmembrane kinase, IRE1, which transduces a lumenal signal imparted by the presence of incorrectly folded proteins to a nuclear event that results in the increased transcription of the previously mentioned genes[11]. Reduced protein synthesis in response to ER stress is associated with polysome disassembly and correlates with increased phosphorylation of eIF2α (refs 1, 3). Phosphorylated eIF2α interferes with the formation of an active 43S translation-initiation complex[12] and expression of a non-phosphorylatable mutant eIF2α (with a mutation of residue S51 to A; S51A) attenuates translational inhibition by ER stress[13]. Two different eIF2α kinases have been identified in mammalian cells, haem-regulated eIF2α kinase (HRI)[14] and the interferon-inducible RNA-dependent protein kinase (PKR)[15]. Ca^{2+} release from ER stores enhances the kinase activity of PKR[16] and dominant-negative mutant forms of PKR attenuate the inhibition of translation mediated by Ca^{2+} release[13]. Although both of these results are consistent with a role for PKR in translational inhibition by ER stress, the level of inhibition of protein synthesis is nearly the same in wild-type and $pkr^{-/-}$ cells (see below). This suggests the existence of alternative eIF2α kinases that may respond to ER stress.

A search of the nucleotide databases (GenBank) identified a *Caenorhabditis elegans* cosmid, CEF46C3, that contains a gene encoding a predicted type I transmembrane protein (one with its amino terminus in the lumen) with a cytoplasmic portion that has a protein-kinase domain most similar to that of PKR and HRI and an external/lumenal domain that shares blocks of identity with *C. elegans* and mammalian Ire1. A human expressed sequence tag (EST) that encodes a peptide fragment with similarity to this *C. elegans* protein was used as a hybridization probe to clone a full-length complementary DNA encoding a murine homologue. The murine protein, of 1,114 residues, is ubiquitously expressed and has the same predicted type I transmembrane topology and ~30% overall identity to the *C. elegans* protein. Its N terminus has blocks of identity with mammalian Ire1, totalling 20% identity, and its carboxy terminus is ~40% identical to PKR (Fig. 1a, b). A C-terminal Myc-tagged derivative of the full-length protein, when expressed in COS-1 cells, co-localized with endogenous ribophorin (an ER-membrane marker; Fig. 1c). Increased mobility of the metabolically labelled protein doublet in immunoprecipitates treated with either endoglycosidase H or peptide *N*-glycosidase F indicates that it is a glycoprotein that is not transported to the distal Golgi complex and resides in the ER. We do not known why there are two species of the protein in the transfected cells (Fig. 1d). On the basis of its similarity to PKR and its localization in the ER membrane, we named the protein PERK (for PKR-like ER kinase). We proposed that PERK, like Ire1, might respond to ER-stress signals and transduce these to changes in protein-synthesis rates through phosphorylation of eIF2α.

The purified kinase domain of PERK, expressed as a fusion protein with glutathione-*S*-transferase (GST) in *Escherichia coli* undergoes autophosphorylation *in vitro* (Fig. 2a). The active kinase phosphorylates purified eIF2α effectively, indicating that the latter is a direct substrate (Fig. 2a, middle). Phosphorylation of eIF2α takes place on S51 (Fig. 2b), the same residue that is targeted by PKR and HRI[12]. Treatment of translation-competent reticulocyte lysate with purified, bacterially expressed PERK leads to a profound inhibition in mRNA translation. Replacing K618 of PERK with alanine (K618A), a mutation that abolishes the ability of the protein to undergo autophosphorylation or to phosphorylate eIF2α (Fig. 2a, lane 2, top and middle), also abolishes its ability to inhibit translation (Fig. 2c, lane 2). Cells transfected with PERK expression plasmids accumulate large quantities of the protein, and when levels of expression reach a certain threshold PERK's kinase activity is activated (Fig. 3c, compare lanes 2, 9). This correlates with profound inhibition of mRNA translation (Fig. 2d, e), indicating that the effects of PERK on protein translation observed *in vitro* in the reticulocyte lysate also occur in cells *in vivo*.

Mouse embryonic fibroblasts that are nullizygous for *pkr* are not impaired in their ability to reduce levels of protein synthesis when treated with agents that induce ER stress (Fig. 3a). This indicates that another eIF2α kinase, perhaps PERK, may be involved in the attenuation of translation under these circumstances. Results of metabolic labelling experiments show that PERK is a phosphoprotein. In response to ER stress, it undergoes hyperphosphorylation and an associated shift in mobility during SDS–polyacrylamide-gel electrophoresis (Fig. 3b). The shift is not observed for the inactive K618A mutant, is reduced by dephosphorylation *in vitro* and correlates with the autokinase activity of immunoprecipitated PERK (Fig. 3b, c). These ER-stress-induced changes in PERK are consistent with intermolecular or intramolecular autophosphorylation, as has been suggested for the activation of the other known eIF2α kinases, HRI[17] and PKR[15]. The stress-activated conformational shift in PERK is independent of synthesis of new PERK protein (Fig. 3c, lane 4), and treatment with arsenite, heat-shock or

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irradiation with ultraviolet light—conditions that trigger a cytoplasmic but not an ER stress response[2]—induce only a minimal shift in PERK's mobility (Fig. 3c, lanes 5–7). Thus the change in conformation is a post-translational modification of PERK that correlates with the ER-stress-dependent activation of the kinase.

The structural similarity between PERK and Ire1 indicates that both proteins may use a similar mechanism to transduce ER stress. This theory is supported by the observation that overexpression of the lumenal domain of PERK interferes with the induction of the UPR marker *chop*[18,19] by ER stress (Fig. 3d). We have found previously that Ire1 activates the *chop* gene and that overexpression of the truncated lumenal domain of Ire1 attenuates *chop* induction by the UPR[10]. Together these results are consistent with a model in which the lumenal domains of Ire1 and PERK share activating upstream signals.

The UPR and translational inhibition by ER stress proceed by

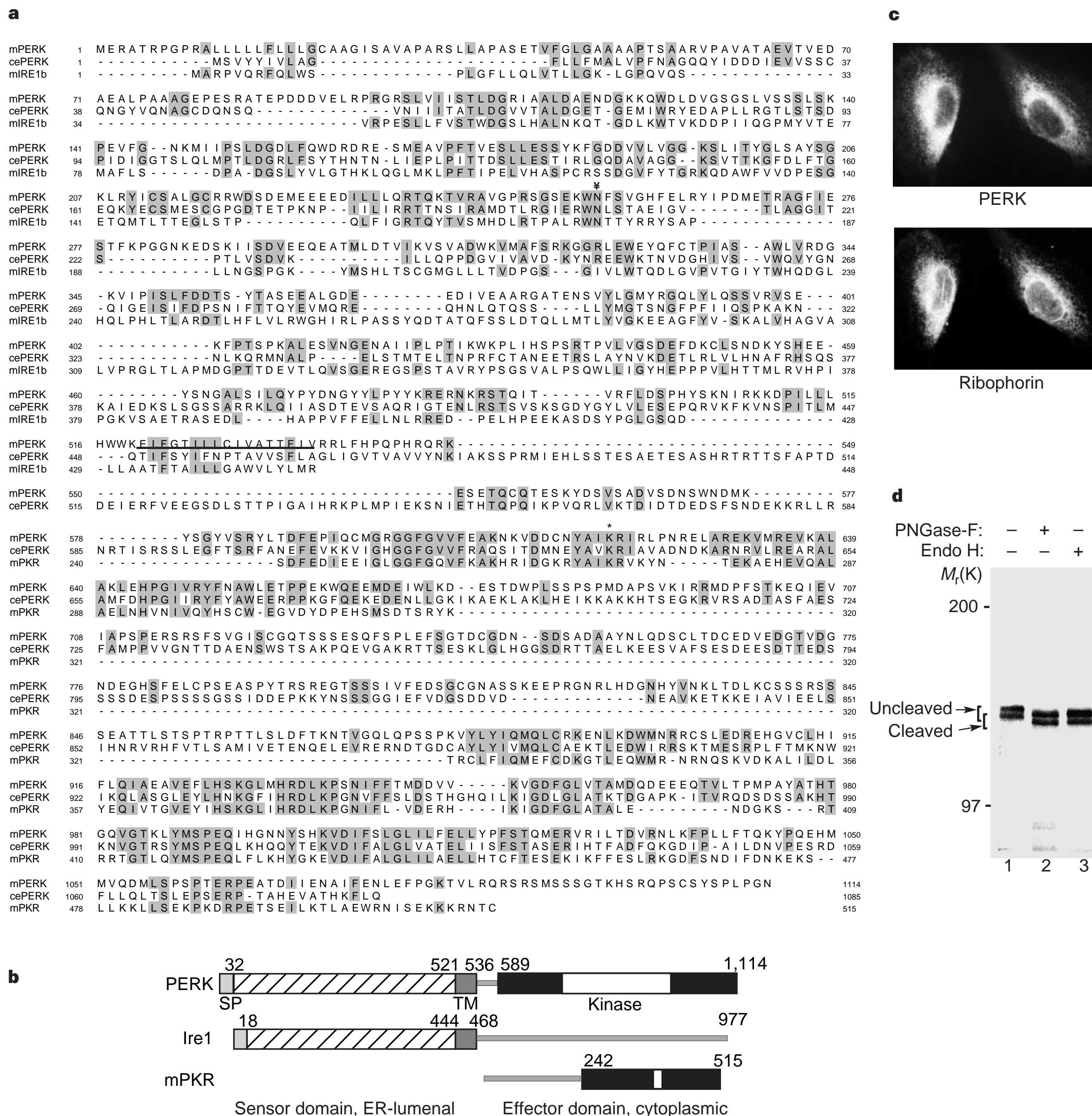

Figure 1 *perk* encodes an ER-resident protein with similarity to Ire1 and PKR. **a**, Alignment of the peptide sequence of mouse PERK, its putative *C. elegans* orthologue, mouse Ire1-β and mouse PKR. Identical residues are shaded. The predicted *N*-linked glycosylation site, conserved in PERK and Ire1-β, is identified by a '¥' symbol. The invariant lysine (in kinase domain 2 of PERK) is marked by an asterisk and the predicted transmembrane domain of PERK is underlined. **b**, The predicted peptide features of PERK, Ire1 and PKR. SP, signal peptide; TM, transmembrane domain. The lumenal domain is cross-hatched and the predicted two lobes of the kinase domain, conserved between PERK and PKR and separated by a large insert in the former (white box), are black. **c**, Photomicrographs of COS-1 cells co-stained for Myc-tagged PERK and endogenous ribophorin (a marker for rough ER). **d**, Autoradiogram of Myc-tagged PERK immunoprecipitated from metabolically labelled COS-1 cells and treated *in vitro* with the glycosidases endoglycosidase H (Endo H) or peptide *N*-glycosidase F (PNGase-F). The migration of the normal (uncleaved) and deglycosylated (cleaved) PERK doublets is indicated.

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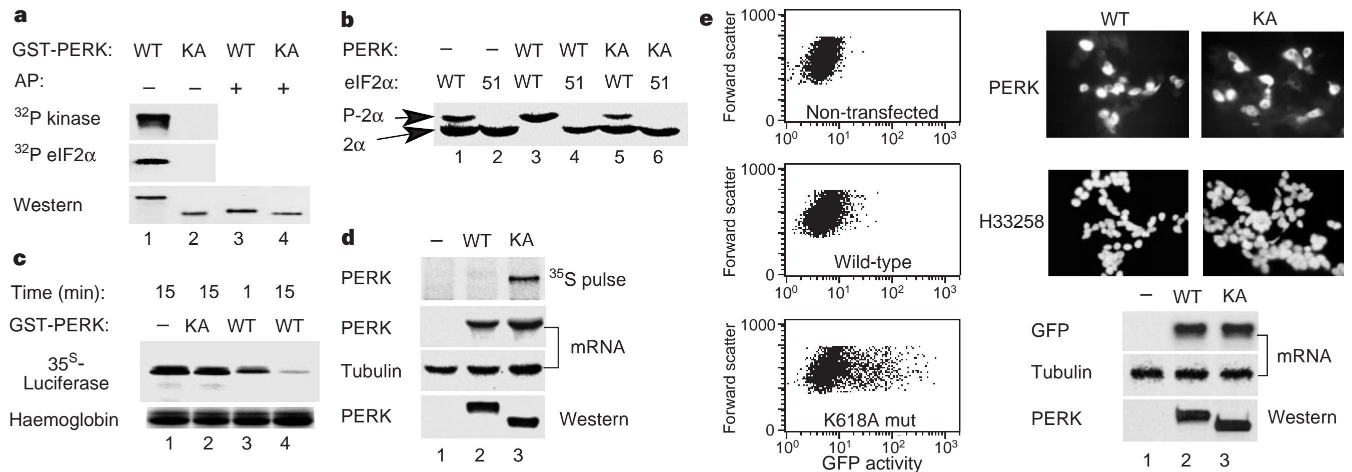

Figure 2 PERK is an eIF2α kinase that inhibits translation. **a**, Top, autoradiogram of the *in vitro* autokinase activity of the wild-type (WT) and K618A mutant (KA) PERK, purified as GST-fusion proteins from *E. coli*. Middle, autoradiogram of eIF2α that had been added to the kinase reaction and immunoprecipitated with a specific antiserum. Bottom, PERK western blot of same lysate untreated or treated with alkaline phosphatase (AP). **b**, Autoradiogram of ^{35}S-labelled wild-type and S51A mutant (51) eIF2α that had been incubated with purified wild-type and mutant PERK. The phosphorylated (p-2α) and non-phosphorylated (2α) forms of eIF2α were resolved by isoelectric focusing. **c**, Autoradiogram of ^{35}S-labelled luciferase translated from mRNA in reticulocyte lysates that had been pre-incubated with purified wild-type and mutant PERK for the indicated times. Haemoglobin staining serves as a loading control. **d**, Reduced synthesis rates of wild-type compared with mutant PERK. Top, autoradiogram of ^{35}S-labelled PERK, immunoprecipitated from pulse-labelled COS-1 cells 48 h after transfection with the indicated PERK expression plasmids. Middle, northern blot of PERK and α-tubulin mRNA from identical plates, showing similar levels of wild-type and mutant PERK mRNA. Bottom, immunoblot showing similar levels of both PERK proteins and revealing that all the wild-type PERK in these cells had been activated. **e**, Interference with translation of a co-expressed green fluorescent protein (GFP) reporter gene by PERK. Cells transfected with a reporter plasmid expressing both GFP and wild-type or K618A mutant PERK were analysed by fluorescence-activated cell sorting for GFP activity (left panels), stained for PERK expression (top right four panels) and analysed for GFP reporter mRNA by northern blot and PERK protein by western blot (lower left panels).

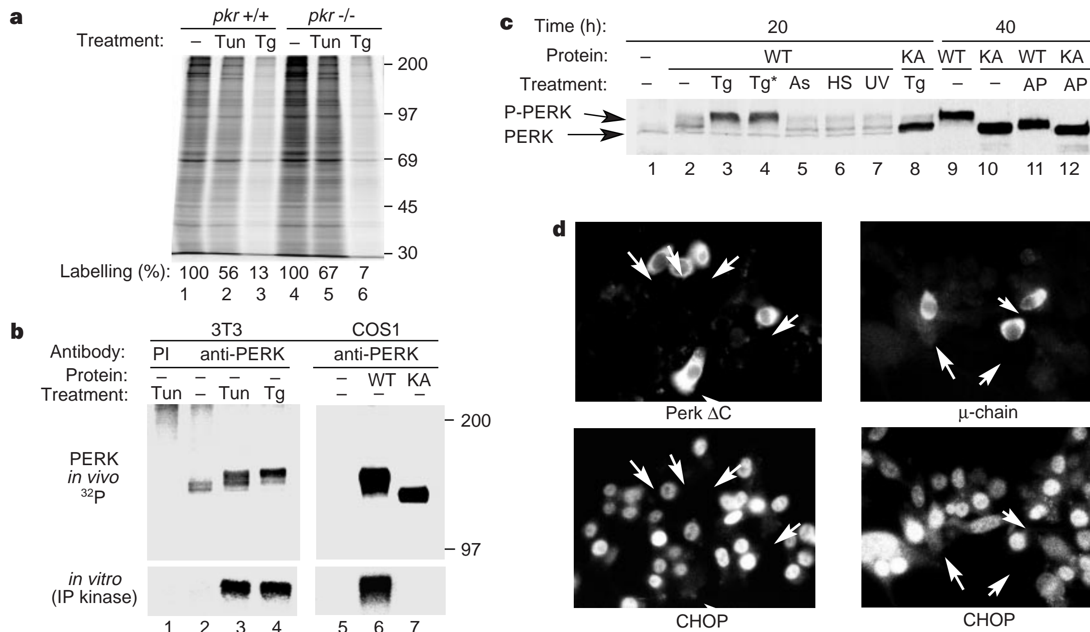

Figure 3 PERK is activated by ER stress. **a**, $pkr^{-/-}$ cells are capable of attenuating translation in response to ER stress. Autoradiogram of total proteins synthesized in wild-type (+/+) and mutant (–/–) mouse embryo fibroblasts that had been untreated or exposed to tunicamycin (Tun) or thapsigargin (Tg) before a 30-min pulse label with ^{35}S-methionine. **b**, Top, autoradiogram of endogenous PERK immunoprecipitated from NIH3T3 cells (lanes 1–4) or of exogenous wild-type or K618A mutant PERK from COS-1 cells transfected with the indicated expression vectors (lanes 5–7). Cells had been preloaded with ^{32}P-labelled orthophosphate and exposed to a brief treatment with tunicamycin or thapsigargin as indicated. Lane 1 was immunoprecipitated with preimmune serum (PI). Bottom, *in vitro* autophosphorylation of PERK immunoprecipitated (IP) from cells treated as above. The left and right panels are exposures of the same gel that differ by a factor of 10 (left > right). **c**, Immunoblot of PERK in lysates of COS-1 cells transfected with wild-type (lanes 2–7, 9, 11) or K618A mutant (lanes 8, 10, 12) PERK and collected 20 (lanes 1–8) or 40 (lanes 9–12) h after transfection. Lanes 3, 4 and 8 were treated with thapsigargin; lane 4 was pretreated with anisomycin (asterisk) to block protein synthesis. As, arsenite; HS, heat-shock; UV, ultraviolet irradiation. Extracts from lanes 11 and 12 were treated with alkaline phosphatase (AP) before being loaded on the gel. P-PERK, phosphorylated PERK on the gel. **d**, Immunostaining of CHOP in tunicamycin-treated NIH3T3 cells. Upper panels, photomicrographs of cells expressing the lumenal domain of PERK (PERKΔC) or the human immunoglobulin μ-chain, a protein that is retained in the ER and serves as a control for the effects of PERKΔC. Lower panels, photomicrographs of the same cells stained with an antibody to CHOP. The arrows point to the cells expressing the exogenous PERKΔC or μ-chain.

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parallel yet distinct pathways[20]. Our findings suggest considerable homology between the two processes: both are initiated by transmembrane ER-resident kinases, Ire1 or PERK, which have similar lumenal domains, and both kinases undergo ER-stress-induced activation, a step that may involve an activating autophosphorylation event[21,22] (Fig. 3). Once activated, however, the two kinases appear to deliver distinct signals. On the basis of comparisons with yeast, mammalian Ire1 probably acquires endonuclease activity to cleave specific mRNA substrates[9] and effect enhanced expression of upstream activators of the UPR[23]. PERK, on the other hand, phosphorylates eIF2α and attenuates translation. Thus, in mammalian cells two signalling pathways that are responsive to ER stress appear to have evolved by appending different effector functions to otherwise similarly structured upstream transducers.

Note added in proof: The rat orthologue of PERK has recently been identified as an elF2α kinase that is expressed at high levels in the pancreas, an organ that is heavily engaged in protein secretion and ER trafficking[26]. This finding underscores the potential physiological significance of PERK's role in coupling folding to translation. □

Methods

Cloning of *Perk* cDNA. We used the insert from human EST clone IMAGE number 746909 as a hybridization probe to screen 3×10^6 clones from an unamplified NIH3T3 fibroblast cDNA library in λ-Zap.express (Stratagene). We identified 19 clones.

Expression plasmids. A C-terminal Myc tag and K618A mutation were incorporated into PERK by altering the *Perk* cDNA by polymerase chain reaction. The modified cDNAs were ligated into the pcDNA1/Amp expression vector (Invitrogen). PERK lacking the C terminus (PERKΔC) was created by an internal *Sna*BI–*Sma*I deletion (removing amino acids 582–1,081 of the protein) of the Myc-tagged full-length cDNA. A bacterial expression plasmid encoding a GST-fusion protein with the cytoplasmic domain of PERK (amino acids 537–1,114) was constructed in pGEX 4T1 (Pharmacia). The purified fusion protein, of relative molecular mass ~93,000 (M_r ~93K), was used in the *in vitro* assays described below and to raise rabbit antisera.

Cell culture and transfection. COS-1, NIH3T3 and $pkr^{+/+}$ and $pkr^{-/-}$ mouse embryonic fibroblasts were maintained in DMEM supplemented with 10% fetal bovine serum. Unless otherwise indicated, cells were treated with 2 μg ml^{-1} tunicamycin for 4 h, 1 μM thapsigargin for 30 min, 100 μM sodium arsenite for 2 h, and heat-shock at 42 °C for 2 h; pretreatment was with 27 J m^{-2} ultraviolet light or 10 μg ml^{-1} anisomycin for 45 min. COS-1 cells were transfected with 3 μg plasmid DNA per 60-cm dish by the DEAE-Dextran method. NIH3T3 cells were transfected with 1 μg DNA per 35-mm dish using Lipofectamine Plus (Life Technologies). Immunoblots were performed with rabbit anti-PERK serum diluted 1:10,000 nearly as described[18] except that cell lysates were prepared in a HEPES–Triton-X100 buffer (see below) and contained 10 μg total protein. Immunostaining was done as described[10].

Cell labelling and immunoprecipitation. Transfected COS-1 cells were starved for 30 min in methionine-free DMEM containing 10% dialysed fetal calf serum, then labelled for 2 h with 250 μCi ml^{-1} ^{35}S-Translabel (ICN) in the same medium. Cells were lysed in RIPA buffer immediately after labelling (Fig. 2d) or after a 4 h cold-chase in complete media (Fig. 1d). The labelled protein was immunoprecipitated with the indicated antibodies and resolved by 6% SDS–PAGE. Where indicated, the immunopurified PERK was digested for 16 h at 37 °C with 0.025 U endoglycosidase H or 0.25 U peptide *N*-glycosidase F according to the manufacturer's instructions (Boehringer). *In vivo* ^{32}P-labelling of PERK was performed by preloading 10-cm plates of NIH3T3 cells with 125 μCi ml^{-1} ^{32}P-orthophosphate for 14 h before stress treatment and lysis in HEPES–Triton-X100 buffer (20 mM HEPES, pH 7.5, 150 mM NaCl, 1% Triton-X100, 10% glycerol, 1 mM EDTA, 10 mM sodium diphosphate, 100 mM NaF, 17.5 mM B-glycerophosphate, 1 mM phenylmethylsulphonyl fluoride, 4 μg ml^{-1} aprotonin, and 2 μg ml^{-1} pepstatin A). Nuclei were removed by brief centrifugation and the extracts were adjusted to a final concentration of 0.5% sodium deoxycholate and 0.1% SDS before immunoprecipitation with anti-PERK antiserum (1 μl).

***In vitro* phosphorylation.** These reactions, containing immunopurified or recombinant bacterially expressed wild-type or K618A mutant GST–PERK (50 ng), were done in 20 μl kinase buffer[24] at 30 °C for 30 min with either 5 μCi [γ-^{32}P]ATP (4,500 Ci $mmol^{-1}$; ICN; Fig. 2a, 3b) or 0.1 mM unlabelled ATP (Fig. 2b). Where indicated, the reactions contained ~10 ng partially purified human eIF2α, which was immunoprecipitated from the supernatant after incubation (Fig. 2a), or 0.5 μl *in vitro*-translated ^{35}S-methionine-labelled wild-type or mutant (S51A) eIF2α (TNT reticulocyte lysate, Promega; Fig. 2b). The products in Fig. 2b were analysed by isoelectric focusing using Pharmalytes, pH 4–6.5, as described[25]. To measure translational inhibition by PERK, glutathione–Sepharose beads (1 μl bed volume) containing ~50 ng GST–PERK (wild-type or K618A) were pre-incubated for 1–15 min at room temperature with 12 μl of an *in vitro* translation mixture (Promega) before addition of 0.5 μg *in vitro*-transcribed capped luciferase mRNA. The labelled luciferase protein was resolved by 10% SDS–PAGE.

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1. Prostko, C. R., Brostrom, M. A. & Brostrom, C. O. Reversible phosphorylation of eukaryotic initiation factor 2 alpha in response to endoplasmic reticular signaling. *Mol. Cell Biochem.* **127–128,** 255–265 (1993).
2. Brostrom, C. O. & Brostrom, M. A. Regulation of translational initiation during cellular response to stress. *Prog. Nucleic Acid Res. Mol. Biol.* **58,** 79–125 (1998).
3. Wong, W. L., Brostrom, M. A., Kuznetsov, G., Gmitter-Yellen, D. & Brostrom, C. O. Inhibition of protein synthesis and early protein processing by thapsigargin in cultured cells. *Biochem. J.* **289,** 71–79 (1993).
4. Lee, A. Mammalian stress response: induction of the glucose-regulated protein family. *Curr. Biol.* **4,** 267–273 (1993).
5. Kozutsumi, Y., Segal, M., Normington, K., Gething, M. J. & Sambrook, J. The presence of malfolded proteins in the endoplasmic reticulum signals the induction of glucose-regulated proteins. *Nature* **332,** 462–464 (1988).
6. Gething, M. J. & Sambrook, J. Protein folding in the cell. *Nature* **355,** 33–45 (1992).
7. Cox, J. S., Shamu, C. E. & Walter, P. Transcriptional induction of genes encoding endoplasmic reticulum resident proteins requires a transmembrane protein kinase. *Cell* **73,** 1197–1206 (1993).
8. Mori, K., Ma, W., Gething, M. J. & Sambrook, J. A transmembrane protein with a cdc2+/CDC28-related kinase activity is required for signaling from the ER to the nucleus. *Cell* **74,** 743–756 (1993).
9. Tirasophon, W., Welihinda, A. A. & Kaufman, R. J. A stress response pathway from the endoplasmic reticulum ot the nucleus requires a novel bifunctional protein kinase/endoribonuclease (Ire1p) in mammalian cells. *Genes Dev.* **12,** 1812–1824 (1998).
10. Wang, X.-Z. *et al.* Cloning of mammalian Ire1 reveals diversity in the ER stress responses. *EMBO J.* **19,** 5708–5717 (1998).
11. Shamu, C. E. Splicing together the unfolded-protein response. *Curr. Biol.* **7,** R67–R70 (1997).
12. Hinnebusch, A. G. The eIF-2 alpha kinases: regulators of protein synthesis in starvation and stress. *Semin. Cell Biol.* **5,** 417–426 (1994).
13. Srivastava, S. P., Davies, M. V. & Kaufman, R. J. Calcium depletion from the endoplasmic reticulum activates the double-stranded RNA-dependent protein kinase (PKR) to inhibit protein synthesis. *J. Biol. Chem.* **270,** 16619–16624 (1995).
14. Chen, J. J. & London, I. M. Regulation of protein synthesis by heme-regulated eIF-2 alpha kinase. *Trends Biochem. Sci.* **20,** 105–108 (1995).
15. Berry, M. J., Knutson, G. S., Lasky, S. R., Munemitsu, S. M. & Samuel, C. E. Mechanism of interferon action. Purification and substrate specificities of the double-stranded RNA-dependent protein kinase from untreated and interferon-treated mouse fibroblasts. *J. Biol. Chem.* **260,** 11240–11247 (1985).
16. Prostko, C. R., Dholakia, J. N., Brostrom, M. A. & Brostrom, C. O. Activation of the double-stranded RNA-regulated protein kinase by depletion of endoplasmic reticular calcium stores. *J. Biol. Chem.* **270,** 6211–6215 (1995).
17. Fagard, R. & London, I. M. Relationship between phosphorylation and activity of heme-regulated eukaryotic initiation factor 2 alpha kinase. *Proc. Natl Acad. Sci. USA* **78,** 866–870 (1981).
18. Wang, X.-Z. *et al.* Signals for the stressed endoplasmic reticulum induce C/EBP homologous protein (CHOP/GADD153). *Mol. Cell. Biol.* **16,** 4273–4280 (1996).
19. Price, B. & Calderwood, S. Gadd45 and Gadd153 messenger RNA levels are increased during hypoxia and after exposure of cells to agents which elevate the levels of glucose-regulated proteins. *Cancer Res.* **52,** 3814–3817 (1992).
20. Brostrom, M. A., Prostko, C. R., Gmitter, D. & Brostrom, C. O. Independent signaling of grp78 gene transcription and phosphorylation of eukaryotic initiator factor 2 alpha by the stressed endoplasmic reticulum. *J. Biol. Chem.* **270,** 4127–4132 (1995).
21. Shamu, C. E. & Walter, P. Oligomerization and phosphorylation of the Ire1p kinase during intracellular signaling from the endoplasmic reticulum ot the nucleus. *EMBO J.* **15,** 3028–3039 (1996).
22. Welihinda, A. A. & Kaufman, R. J. The unfolded protein response pathway in *Saccharomyces cerevisiae*. Oligomerization and trans-phosphorylation of Ire1p (Ern1p) are required for kinase activation. *J. Biol. Chem.* **271,** 18181–18187 (1996).
23. Sidrauski, C. & Walter, P. The transmembrane kinase Ire1p is a site-specific endonuclease that initiates mRNA splicing in the unfolded protein response. *Cell* **90,** 1031–1039 (1997).
24. Wu, S. & Kaufman, R. J. A model for the double-stranded RNA (dsRNA)-dependent dimerization and activation of the dsRNA-activated protein kinase PKR. *J. Biol. Chem.* **272,** 1291–1296 (1997).
25. Savinova, O. & Jagus, R. Use of vertical slab isoelectric focusing and immunoblotting to evaluate steady-state phosphorylation of eIF2 alpha in cultured cells. *Methods* **11,** 419–425 (1997).
26. Shi, Y. *et al.* Identification and characterization of pancreatic eukaryotic initiation factor 2 alpha-subunit kinase, PEK, involved in translational control. *Mol. Cell. Biol.* **18,** 7499–7509 (1998).

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