Research

The eIF2 α kinase PERK limits the expression of hippocampal metabotropic glutamate receptor-dependent long-term depression

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The proper regulation of translation is required for the expression of long-lasting synaptic plasticity. A major site of translational control involves the phosphorylation of eukaryotic initiation factor 2 α (eIF2 α) by PKR-like endoplasmic reticulum (ER) kinase (PERK). To determine the role of PERK in hippocampal synaptic plasticity, we used the *Cre-lox* expression system to selectively disrupt PERK expression in the adult mouse forebrain. Here, we demonstrate that in hippocampal area CA1, metabotropic glutamate receptor (mGluR)-dependent long-term depression (LTD) is associated with increased eIF2 α phosphorylation, whereas stimulation of early- and late-phase long-term potentiation (E-LTP and L-LTP, respectively) is associated with decreased eIF2 α phosphorylation. Interesting, although PERK-deficient mice exhibit exaggerated mGluR-LTD, both E-LTP and L-LTP remained intact. We also found that mGluR-LTD is associated with a PERK-dependent increase in eIF2 α phosphorylation. Our findings are consistent with the notion that eIF2 α phosphorylation is a key site for the bidirectional control of persistent forms of synaptic LTP and LTD and suggest a distinct role for PERK in mGluR-LTD.

De novo synthesis of proteins is critical for the expression of long-lasting synaptic plasticity (Kelleher et al. 2004; Costa-Mattioli et al. 2009; Richter and Klann 2009). Protein synthesis is highly regulated at the level of initiation by numerous translational control molecules, including the eukaryotic initiation factor 2 (eIF2) (Sonenberg and Dever 2003). Although phosphorylation of the α subunit of eIF2 inhibits general translation, it selectively stimulates the translation of the transcriptional modulator activating transcription factor 4 (ATF4) (Vattem and Wek 2004), which plays a role as a repressor of cAMP response element-binding protein (CREB)-mediated synaptic plasticity (Bartsch et al. 1995; Abel et al. 1998; Chen et al. 2003). Previously, it was shown that reduction of eIF2 α phosphorylation in mice lacking the eIF2 α kinase general control nonderepressible 2 (GCN2) and in heterozygous knockin mice with a mutation on serine 51 of eIF2 α results in a lowered threshold for inducing long-lasting late-phase long-term potentiation (L-LTP) (Costa-Mattioli et al. 2005, 2007). In addition, mice harboring a deletion of the double-stranded (ds) RNA-activated protein kinase (PKR) show a similar decrease in threshold for inducing L-LTP (Zhu et al. 2011). Conversely, increased eIF2 α phosphorylation in transgenic mice overexpressing PKR causes increased expression of ATF4 and impaired L-LTP (Jiang et al. 2010). Collectively, these findings suggest that the proper regulation of eIF2 α phosphorylation is required for normal synaptic plasticity.

The PKR-like ER kinase (PERK) is a highly conserved protein kinase that phosphorylates eIF2 α to mediate translational control in response to ER stress (Shi et al. 1998; Harding et al. 2000). Global deletion of PERK in mice impairs development of the skeletal system, postnatal growth, and pancreatic viability (Harding et al. 2001; Zhang et al. 2002; Wei et al. 2008). In humans, mutations of the PERK gene (*EIF2AK3*) cause Wolcott-Rallison syndrome (WRS), a rare autosomal recessive disorder characterized by early onset diabetes, liver dysfunction, and pancreas insufficiency (Delepine et al. 2000; Rubio-Cabezas et al. 2009; Julier and Nicolino 2010) and, in some cases, mental retardation (Thornton et al. 1997; Delepine et al. 2000; Senee et al. 2004; Reis et al. 2011). Together, these analyses suggest that PERK functions as a critical modulator of a number of cellular processes requiring precise translational control.

Here, we show that PERK is required for the proper expression of protein synthesis-dependent mGluR-LTD, but not L-LTP, at Schaffer-collateral CA1 synapses in hippocampal slices. Moreover, we observed that LTP-inducing stimulation decreases eIF2 α phosphorylation, whereas mGluR-LTD is associated with a PERK-dependent increase in eIF2 α phosphorylation, demonstrating for the first time that a translation factor is divergently regulated during different protein synthesis-dependent forms of synaptic plasticity. Taken together, our findings suggest that PERK distinctly controls the regulation of eIF2 α phosphorylation during mGluR-dependent LTD in the hippocampus.

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Results

PERK is expressed in the mouse hippocampus and forebrain-specific disruption of PERK preserves hippocampal morphology

To determine whether PERK is expressed in the mouse hippocampus, we incubated coronal brain sections from 4-wk-old wild-type mice with an antibody specific to PERK and counterstained with diaminobenzidine (DAB). We observed that PERK is abundantly expressed in various regions of the brain, including the dentate gyrus, CA1, and CA3 regions of the hippocampus (Fig. 1A). To determine the subcellular localization and distribution of PERK in hippocampal neurons, we stained cultured hippocampal pyramidal neurons (DIV 9) for PERK and the endogenous dendritic marker, MAP2. We found that PERK staining was present in the cell body and colocalized with MAP2-positive dendrites (Fig. 1B). Similarly, we found that PERK also colocalized with eIF2 α in these regions (Fig. 1B). To evaluate the role of PERK in translation-dependent forms of synaptic plasticity in the hippocampus, we employed *Cre-lox* recombination techniques to generate mice in which PERK activity is disrupted specifically in the adult forebrain (PERK cKO) (Trinh et al. 2012). Western blotting confirmed efficient *Cre*-mediated reduction of PERK in hippocampal area CA1, which was correlated with decreased levels of phosphorylated eIF2 α and ATF4 (Fig. 1C). Despite the reduction of PERK in this region, Nissl-stained sections revealed that PERK cKO mice did not demonstrate gross alterations in hippocampal morphology compared to wild-type mice (Fig. 1D). Together, these findings indicate that PERK is expressed in the soma and dendrites of mouse hippocampal neurons and that a forebrain-specific disruption of PERK at 2–3 wk of age does not dramatically alter hippocampal morphology.

PERK cKO mice exhibit normal basal synaptic transmission and LTP

We next investigated whether the disruption of PERK altered hippocampal plasticity by recording fEPSPs in area CA1 of hippocampal slices from PERK mutant and their wild-type littermates. We first examined basal synaptic function in 4- to 5-wk-old mice by eliciting synaptic responses with a range of stimulus intensities and observed similar synaptic input–output relationships in slices from wild-type and PERK cKO mice (Fig. 2A). We next examined paired-pulse facilitation (PPF), a calcium-dependent form of presynaptic plasticity. Similar to the input–output functions, we observed no significant difference in PPF between the two genotypes (Fig. 2B). Together, these findings dem-

Figure 1. Localization of PERK in hippocampal neurons and characterization of PERK cKO mice. (A) PERK is highly expressed in the cell body layer of hippocampal area CA1. Representative light micrograph (4 $\times$ magnification) is shown to the left and the boxed-in area is shown at higher powers (10 $\times$ and 40 $\times$ magnification) to the right. (B) PERK is also present in dendrites of hippocampal pyramidal neurons. (Top panel) Representative confocal images show dual staining for PERK (green) and the dendritic marker MAP2 (red). (Lower panel) Hippocampal pyramidal neurons costained with PERK (green) and total eIF2 α (t-eIF2 α , red). DAPI nuclear stain is shown in blue. Merge of immunofluorescence staining is shown in yellow. Scale bar, 25 μ m. (C) Reduction of PERK expression in hippocampal area CA1 of PERK cKO mice is correlated with reduced levels of phosphorylated eIF2 α and ATF4. (Left panel) Representative Western blots showing PERK, phospho-eIF2 α (p-eIF2 α), and ATF4 in the hippocampus and hippocampal subregions from wild-type (WT) and PERK cKO (KO) mice. (Right panel) Quantification of PERK, eIF2 α phosphorylation, and ATF4 immunoreactivity. WT, $n = 5$; cKO, $n = 5$ [*] $P < 0.05$, [**] $P < 0.01$, [***] $P < 0.005$, one-way ANOVA). All data represent mean values $\pm$ SEM (for all figures). (D) Representative Nissl-staining of hippocampal cell bodies in slices obtained from wild-type (left) and PERK cKO (right) mice show no difference in gross morphology.

onstrate that genetic disruption of the eIF2 α kinase PERK does not exert an adverse effect on either basal synaptic transmission or short-lasting presynaptic plasticity. We proceeded to examine LTP in area CA1 of hippocampal slices from PERK mutant and wild-type mice. To our surprise, we found no significant differences in either early phase LTP (E-LTP) or L-LTP between PERK cKO mice and their wild-type littermates (Fig. 2C,D). These findings indicate that genetic disruption of the eIF2 α kinase PERK in hippocampal neurons has no effect on the expression of either E-LTP or L-LTP.

Figure 2. Basal synaptic transmission and early- and late-phase long-term potentiation (LTP) are normal in PERK cKO mice. (A) Input–output relationships plotted as fEPSP slope vs. fiber volley amplitude show no difference in basal synaptic transmission between genotypes. WT, $n = 11$; cKO, $n = 9$ ($P > 0.05$, two-way ANOVA). (B) Paired-pulse facilitation (PPF) is unaltered in PERK cKO mice compared to their wild-type (WT) littermates. WT, $n = 11$; cKO, $n = 9$ ($P > 0.05$, two-way ANOVA). The percentage of facilitation is shown at interstimulus intervals ranging from 10 to 300 msec. (C) Early-phase LTP evoked by a single 100-Hz train (1 sec) was unaltered in PERK cKO slices compared to WT slices. WT, $n = 7$; cKO, $n = 7$ ($P > 0.05$, measured 5 min before and 30 min after HFS for both genotypes, two-way ANOVA). (D) Late-phase LTP evoked by four trains of HFS was normal in both genotypes. WT, $n = 10$; cKO, $n = 11$ ($P > 0.05$, measured 5 min before and 1.5 h after HFS, two-way ANOVA). Representative traces are shown in each panel.

PERK cKO mice display enhanced mGluR-LTD that is protein synthesis-dependent

Activation of metabotropic glutamate receptor (mGluR)-dependent signaling is tightly coupled to the regulation of translation in hippocampal neurons (Huber et al. 2000; Aschrafi et al. 2005; Banko et al. 2006; Ronesi and Huber 2008). Because the regulation of translation initiation involves eIF2 α phosphorylation by PERK, we determined whether genetic disruption of PERK in the hippocampus impacted mGluR-LTD. Hippocampal slices from PERK cKO and wild-type mice were incubated with DHPG (50 μ M, 10 min) to induce protein synthesis-dependent mGluR-LTD. In contrast to our LTP studies, PERK cKO slices exhibited enhanced mGluR-LTD compared to their wild-type littermates (Fig. 3A). Similarly, another paradigm of mGluR-LTD induced by paired-pulse low-frequency stimulation (PP-LFS) in the presence of D(-)-2-amino-5-phosphonopentanoic acid (D-APV) (Huber et al. 2000), was enhanced in slices from PERK cKO mice when compared to wild-type mice (Fig. 3B). Together, these findings suggest that PERK-directed eIF2 α phosphorylation is required for the normal expression of chemically and electrically induced mGluR-LTD.

As mentioned above, group I mGluR activation elicits a persistent form of LTD in wild-type mice that requires de novo protein synthesis (Huber et al. 2000; Hou and Klann 2004; Ronesi and Huber 2008). In contrast, in *Fmr1* KO mice that lack the translational repressor fragile X mental retardation protein (FMRP), this mGluR-induced LTD is enhanced and independent of new protein synthesis (Hou et al. 2006; Nosyreva and Huber 2006;

Sharma et al. 2010). Therefore, we investigated whether the enhanced mGluR-LTD in the PERK cKO mice required de novo protein synthesis. Unlike mGluR-LTD in *Fmr1* knockout mice, we found that the LTD in the PERK cKO mice was blocked with application of the general protein synthesis inhibitor anisomycin (20 μ M) (Fig. 3C). These results demonstrate that de novo protein synthesis is required for the enhanced mGluR-LTD in PERK cKO mice. Furthermore, we found that NMDA receptor-dependent LTD (NMDAR-LTD) was similar between wild-type and PERK cKO slices (Fig. 3D). These results suggest a specific role for PERK-dependent translational control mechanisms in mGluR-dependent LTD vs. NMDAR-LTD.

Differential regulation of eIF2 α phosphorylation following stimulation that induces LTP and mGluR-LTD

To better understand the regulation of eIF2 α phosphorylation during persistent changes in synaptic strength, we first investigated the effects of LTP-inducing stimulation on eIF2 α phosphorylation in hippocampal slices. Protein synthesis-dependent increases in synaptic strength, induced by tetanic stimulation and with application of either brain-derived neurotrophic factor (BDNF) or the cyclic AMP (cAMP) activator forskolin, have been shown to decrease the phosphorylation of eIF2 α (Takei et al. 2001; Costa-Mattioli et al. 2005). Consistent with these findings, we found that phosphorylation of eIF2 α was reduced following both one and four trains of high-frequency stimulation (HFS) that induces E-LTP and L-LTP, respectively (Fig. 4A). These results indicate that increases in synaptic strength are associated with a reduction of eIF2 α phosphorylation in hippocampal area CA1.

To determine whether a protein synthesis-dependent decrease in synaptic strength also altered eIF2 α phosphorylation, we next examined whether activation of group I mGluRs, which induces LTD in the hippocampus, modulated eIF2 α phosphorylation. We treated hippocampal slices from wild-type mice with DHPG (50 μ M, 10 min) or vehicle (control), and then measured the levels of phosphorylated eIF2 α in homogenates from area CA1. Interestingly, we found that treatment of hippocampal slices with DHPG significantly increased eIF2 α phosphorylation (Fig. 4B). Thus, our findings indicate that an mGluR- and protein synthesis-dependent decrease in synaptic strength promotes eIF2 α phosphorylation in hippocampal area CA1. Collectively, these findings provide evidence for the differential regulation of eIF2 α phosphorylation during long-lasting LTD and LTP.

mGluR-induced increase in eIF2 α phosphorylation is dependent on PERK

As mentioned earlier, mGluR-LTD requires local protein synthesis (Huber et al. 2000). Because the expression of mGluR-LTD was

Figure 3. PERK cKO mice express enhanced mGluR-LTD that is protein synthesis-dependent. (A) DHPG application (50 μ M, 10 min) induced mGluR-LTD in wild-type (WT) slices that was enhanced in PERK cKO slices. WT, $n = 8$ slices; cKO, $n = 8$ slices; seven mice per genotype ($P < 0.01$, two-way ANOVA followed by Bonferroni's post hoc test). (B) mGluR-LTD induced by paired-pulse low-frequency stimulation (PP-LFS) (1-Hz, 50-msec interstimulus interval, 20 min) in the presence of D-APV (50 μ M) was enhanced in PERK cKO slices. WT, $n = 9$ slices; cKO, $n = 7$ slices ($P < 0.05$, two-way ANOVA followed by Bonferroni's post hoc test). (C) Measurement of mGluR-LTD in slices from PERK cKO and WT littermates in the presence of anisomycin (40 μ M) 20 min prior to, during, and 30 min after DHPG (50 μ M, 10 min) treatment indicates that enhanced mGluR-LTD in PERK cKO slices is sensitive to anisomycin. WT, $n = 8$ slices; cKO, $n = 8$ slices; seven mice per genotype ($P > 0.05$, two-way ANOVA). (D) NMDA receptor-dependent LTD induced by paired-pulse low-frequency stimulation (PP-LFS) (1-Hz, 50-msec interstimulus interval, 15 min) was not altered in PERK cKO slices compared with WT slices. WT, $n = 9$ slices; cKO, $n = 12$ slices ($P > 0.05$, two-way ANOVA).

enhanced in PERK cKO mice, we examined whether the LTD-associated increase in eIF2 α phosphorylation was still present in slices from PERK cKO mice. In hippocampal slices from wild-type mice, DHPG-induced LTD was associated with an increase in eIF2 α phosphorylation 10 min after DHPG application (Fig. 4C). Notably, the enhanced level of phosphorylated eIF2 α was blocked following LTD induction in the PERK cKO mice (Fig. 4C). Neither mGluR1 nor mGluR5 expression were altered in hippocampal lysates from PERK cKO mice compared to wild-type littermates, suggesting that the enhanced mGluR-LTD in the PERK cKO mice is not due to increased mGluR-dependent signaling (Fig. 4D). Taken together, these findings support the idea that DHPG induces PERK-dependent phosphorylation of eIF2 α , which normally limits mGluR-LTD.

To determine whether the enhanced mGluR-LTD in the PERK cKO slices is due to changes in de novo protein synthesis, we next examined whether activation of group I mGluRs by DHPG induced de novo protein synthesis. To measure protein synthesis, we used SUNSET (Schmidt et al. 2009), a nonradioactive puromycin end-labeling assay. Following puromycin treatment, we incubated hippocampal slices from 3-mo-old wild-type and PERK cKO mice with DHPG (100 μ M, 10 min) or vehicle (control), and measured the levels of protein synthesis in lysates from area CA1. We found that DHPG caused an increase in de novo protein synthesis in the PERK cKO slices that appeared to be the same magnitude as was observed in wild-type slices (Fig. 5A,B). These

findings suggest that the enhanced mGluR-LTD in PERK cKO slices is due to changes in transcript-specific translation rather than changes in general protein synthesis.

Discussion

Multiple lines of evidence strongly support the notion that tight regulation of translational control is critical for the expression of hippocampal mGluR-LTD (Costa-Mattioli et al. 2009; Richter and Klann 2009; Waung and Huber 2009). First, translation is required as several general protein synthesis inhibitors block mGluR-LTD (Huber et al. 2000). Second, pharmacological and genetic disruption of PI3K, mTORC1, and ERK signaling pathways alter the phosphorylation of multiple initiation factors and prevent the expression of normal mGluR-LTD (Gallagher et al. 2004; Hou and Klann 2004; Banko et al. 2006; Ronesi and Huber 2008). For example, mice lacking 4E-BP2, a translational repressor of eIF4F complex assembly, display enhanced mGluR-LTD (Banko et al. 2006), suggesting that repression of translation initiation normally limits the magnitude of mGluR-LTD. Consistent with these findings, we found that a reduction of eIF2 α phosphorylation by genetic inactivation of PERK resulted in an enhancement of mGluR-dependent LTD (Fig. 3A). Thus, these findings suggest that, similar to 4E-BP2, PERK normally modulates the level of long-lasting depression following the activation of group I mGluRs. It should be noted that despite the lack of

an increase in eIF2 α phosphorylation in the PERK cKO mice following induction of mGluR-LTD, it is possible that the effects of PERK deletion on LTD could be independent of eIF2 α and translation. However, the most parsimonious explanation for the present findings is that PERK-dependent phosphorylation of eIF2 α is required for translational control that limits mGluR-LTD in the hippocampus.

Previous studies demonstrated that mice lacking the translational regulator FMRP exhibit enhanced hippocampal mGluR-LTD and are insensitive to protein synthesis inhibitors (Huber et al. 2002; Hou et al. 2006; Nosyreva and Huber 2006). In contrast, in our LTD studies we found that the enhanced mGluR-LTD in PERK cKO mice were sensitive to protein synthesis inhibition (Fig. 3C), more in keeping with mGluR-LTD in wild-type mice (Huber et al. 2000). One possible explanation for these differences is that FMRP and PERK are acting to trigger distinct proteomic changes during mGluR-dependent LTD.

In this study, we found that DHPG-induced mGluR-LTD triggered an increase in phosphorylated eIF2 α , which was prevented in the absence of PERK (Fig. 4C). As noted above, phosphorylation of eIF2 α inhibits general translation while preferentially stimulating the gene-specific translation of mRNAs with upstream open reading frame (uORF) sequences such as ATF4. Therefore, we hypothesize that PERK normally either decreases general protein synthesis or increases the translation of specific synaptic mRNAs to limit mGluR-LTD. Future studies investigating the identities

Figure 4. PERK is required for the distinct regulation of eIF2 α phosphorylation following mGluR-LTD induction. (A) E-LTP and L-LTP stimulation decreases eIF2 α phosphorylation in hippocampal area CA1. Untetanized (control) slices vs. 1×100 Hz (E-LTP) and 4×100 Hz (L-LTP) slices are compared ($n = 5$ slices per condition, [*] $P < 0.05$, [**] $P < 0.01$, one-way ANOVA). (B) DHPG application ($50 \mu\text{M}$, 10 min) increases eIF2 α phosphorylation in hippocampal area CA1 ($n = 6$ slices per condition, [*] $P < 0.05$, Student's t -test). (C) The DHPG-induced increase in eIF2 α phosphorylation in WT slices is blocked in PERK cKO slices. Hippocampal slices from PERK cKO and WT mice were treated with or without DHPG ($50 \mu\text{M}$, 10 min, $n = 6$ slices per condition, [*] $P < 0.05$, one-way ANOVA). (D) mGluR1 and mGluR5 expression were unaltered in hippocampal lysates from PERK cKO mice compared to wild-type littermates (mGluR1, $n = 4$, $P = 0.6$; mGluR5, $n = 6$, $P = 0.8$, Student's t -test). Representative Western blots are shown in each panel.

of the newly synthesized LTD proteins following synaptic activation of mGluRs in these mice should provide insight into the different regulatory mechanisms that control mGluR-LTD.

We observed that eIF2 α phosphorylation was differentially regulated upon activation of LTP and LTD (Fig. 4A,B). These findings suggest that distinct pools of eIF2 α are phosphorylated and mRNAs are translated during NMDA-receptor dependent LTP and mGluR-LTD. In support of this notion, a recent study showed that DHPG stimulation causes preferential recruitment of certain locally translated mRNAs, without alterations to the size or number of translational hotspots (Kim et al. 2013). Moreover, mGluR-LTD, which is enhanced in PERK cKO mice (Fig. 3A), was unaffected in mice lacking the eIF2 α kinase GCN2 (Costa-Mattioli et al. 2005). In contrast, L-LTP, which had a lowered threshold for induction in GCN2-deficient mice (Costa-Mattioli et al. 2005), was unaffected in PERK cKO mice (Fig. 2C,D). Thus, it appears that GCN2-dependent translational control is specific to NMDA receptor-dependent LTP, whereas PERK-dependent translational control is specific to mGluR-LTD. Moreover, our findings provide the first direct evidence of a translation factor that is differentially regulated by protein synthesis-dependent LTP and LTD and indicate a distinct role for the proper regulation of eIF2 α by PERK during mGluR-dependent LTD.

Precise translational control mechanisms in dendrites are considered critical for long-lasting plasticity of synaptic connec-

tions and for the expression of cognitive functions that rely on such plasticity (Kelleher et al. 2004; Sutton and Schuman 2006; Costa-Mattioli et al. 2009; Richter and Klann 2009). Consistent with this, we recently reported that disruption of PERK-dependent translational control mechanisms in the prefrontal cortex of PERK cKO mice results in multiple cognitive impairments, including severe behavioral inflexibility (Trinh et al. 2012). In the present study, our findings suggest that PERK-directed translational control of eIF2 α phosphorylation distinctly limits the expression of mGluR-dependent LTD in the hippocampus. Based on these results, we speculate that disruption of PERK-dependent phosphorylation of eIF2 α following activation of group I mGluRs may serve as a mechanism for the behavioral manifestations observed in PERK-deficient mice. Future studies investigating the specific role of PERK-regulated translation in the brain will prove useful to elucidate the molecular underpinnings of normal cognitive processes and how they are altered in neurological disorders and neurodegenerative diseases.

Materials and Methods

Materials

Alexa Fluor 488-coupled goat anti-rabbit, Alexa Fluor 568-coupled goat anti-mouse, and anti-rabbit phospho-eIF2 α ⁵⁵² antibodies were from Invitrogen. Rabbit anti-PERK-like endoplasmic reticulum kinase (PERK) antibody was from Rockland. Mouse anti-eIF2 α (L57A5), and rabbit anti-PERK (C33E10) and rabbit anti-mGluR1 antibodies were from Cell Signaling Technology. Anti-puromycin (12D10) antibody was kindly provided by Dr. P. Pierre. Anti-mGluR5 antibody was from R&D Systems. 3,3'-Diaminobenzidine (DAB) substrate staining kit was from Vector Laboratories. (RS)-3,5-Dihydroxyphenylglycine (DHPG) and anisomycin were obtained from Tocris Bioscience. Puromycin (P8833) and mouse anti-actin and anti-MAP2 antibodies were from Sigma.

Animals

Transgenic mice containing a forebrain-specific deletion of PERK (*CamkIIa-Cre; Perk^{loxP/loxP}*) were generated, maintained, and genotyped as described previously (Trinh et al. 2012).

Figure 5. DHPG-induced mGluR-LTD in slices from PERK cKO mice is associated with an increase of de novo protein synthesis similar to that in wild-type mice. (A) Representative Western blot of lysates from wild-type (WT) and PERK cKO hippocampal slices treated with puromycin ($5 \mu\text{g}/\text{mL}$, 35 min) and incubated with either ACSF (Cntl) or DHPG ($100 \mu\text{M}$) for 10 min to measure protein synthesis at baseline levels and in response to DHPG treatment. (B) Cumulative graph with percentages expressed relative to WT control (Cntl), $n = 3$ mice per genotype, 2–3 slices per condition ([*] $P < 0.05$, Student's t -test). All data represent mean values $\pm$ SEM.

Immunohistochemistry and Nissl staining

Immunohistochemical and Nissl staining techniques were performed using standard procedures as described previously (Banko et al. 2006). For immunohistochemical studies, sections from 4-wk-old wild-type mice were incubated with rabbit anti-PERK (Rockland, 1:200) followed by secondary antibody incubation and detection using the DAB staining method. For immunofluorescence studies, mouse primary hippocampal neurons (DIV 9) were incubated with rabbit anti-PERK (Rockland, 1:200) and either mouse anti-MAP2 (1:500) or mouse anti-eIF2 α (1:500) antibodies followed by an incubation with Alexa Fluor 488-coupled goat anti-rabbit and Alexa Fluor 568-coupled goat anti-mouse antibodies (1:500).

Hippocampal slice preparations and treatment

Transverse hippocampal slices were prepared and CA1 regions were dissected as described previously (Banko et al. 2005). Briefly, for mGluR-LTD experiments, slices from 4- to 5-wk old mice were equilibrated in oxygenated artificial CSF (ACSF) and subsequently incubated with DHPG (50 μ M) for 10 min prior to being snap-frozen over dry ice. For LTP studies, slices were isolated and prepared from 3- to 4-mo-old adult mice. Upon stimulation of E-LTP and L-LTP (see Electrophysiology), slices were prepared in a fashion identical to that used for mGluR-LTD experiments. For NMDA receptor-dependent LTD studies, slices were isolated and prepared from 3-mo-old mice.

Electrophysiology

Field excitatory postsynaptic potentials (fEPSPs) were recorded from hippocampal slices as described previously (Banko et al. 2005). For mGluR-LTD studies, slices were incubated with DHPG (50 μ M) for 10 min in the presence of either vehicle or anisomycin (20 μ M). For PP-LFS studies, mGluR-LTD was induced in the presence of D-(–)-2-amino-5-phosphonopentanoic acid (D-APV) (50 μ M, Sigma) by using 900 paired-pulse stimuli with a 50-msec interstimulus interval delivered at 1 Hz for 20 min. NMDA receptor-dependent LTD was induced by 900 paired-pulse stimulations with 50-msec interstimulus interval delivered at 1 Hz for 15 min (Jiang et al. 2007). For LTP studies, LTP was induced with either one train (E-LTP) or four trains (L-LTP) (5-min intertrain interval [ITI]) of a 100-Hz high-frequency stimulation (HFS) for 1 sec.

Western blotting

Western blots were performed using standard procedures as described previously (Banko et al. 2005). Notably, for optimal detection of PERK in mouse brain samples, a minimum of 40 μ g of protein was needed. Primary antibodies of interest include: PERK (C33E10, 1:500), phospho-eIF2 α ^{SS2} (1:1000), eIF2 α (1:1000), ATF4 (1:2000), mGluR1 (1:1000), mGluR5 (1:200), and actin (1:10000).

Measurement of de novo protein synthesis

Four-hundred-micrometer transverse hippocampal slices were obtained from 3-mo-old wild-type and PERK cKO mice. Puromycin labeling of the slices was adapted from procedures described previously (Schmidt et al. 2009). Briefly, slices were allowed to recover in ACSF at 32°C for 2 h and subsequently treated with puromycin (5 μ g/mL) for 35 min and incubated with either DHPG (100 μ M) or ACSF vehicle (control) for 10 min. Reactions were stopped by flash freezing the slices on dry ice. Then, proteins were prepared, blotted, and quantified by standard Western blotting procedures. Fifty micrograms of puromycin-labeled protein were resolved on 4%–12% gradient gels and visualized using an antibody specific to puromycin. Protein synthesis levels were determined by taking the total lane signal from 30 kDa to 250 kDa and subtracting the signal from a control lane that was not labeled by puromycin. Comparisons of protein synthesis levels between both genotypes and treatment were made by normalizing to the average wild-type control signal obtained from different experimental replicates.

Statistical analysis

All data are presented as the mean $\pm$ SEM. Intergroup differences were evaluated by a one-way ANOVA. For electrophysiological data analysis, two-way ANOVA and post-hoc tests were used where appropriate. When only two means were analyzed, a two-tailed Student's *t*-test analysis was performed. Values of *P* < 0.05 were considered to represent statistically significant differences.

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References

- Abel T, Martin KC, Bartsch D, Kandel ER. 1998. Memory suppressor genes: Inhibitory constraints on the storage of long-term memory. *Science* **279**: 338–341.
- Aschrafi A, Cunningham BA, Edelman GM, Vanderklish PW. 2005. The fragile X mental retardation protein and group I metabotropic glutamate receptors regulate levels of mRNA granules in brain. *Proc Natl Acad Sci* **102**: 2180–2185.
- Banko JL, Poulin F, Hou L, DeMaria CT, Sonenberg N, Klann E. 2005. The translation repressor 4E-BP2 is critical for eIF4F complex formation, synaptic plasticity, and memory in the hippocampus. *J Neurosci* **25**: 9581–9590.
- Banko JL, Hou L, Poulin F, Sonenberg N, Klann E. 2006. Regulation of eukaryotic initiation factor 4E by converging signaling pathways during metabotropic glutamate receptor-dependent long-term depression. *J Neurosci* **26**: 2167–2173.
- Bartsch D, Ghirardi M, Skehel PA, Karl KA, Herder SP, Chen M, Bailey CH, Kandel ER. 1995. *Aplysia* CREB2 represses long-term facilitation: Relief of repression converts transient facilitation into long-term functional and structural change. *Cell* **83**: 979–992.
- Chen A, Muzzio IA, Malleret G, Bartsch D, Verbitsky M, Pavlidis P, Yonan AL, Vronskaya S, Grody MB, Cepeda I, et al. 2003. Inducible enhancement of memory storage and synaptic plasticity in transgenic mice expressing an inhibitor of ATF4 (CREB-2) and C/EBP proteins. *Neuron* **39**: 655–669.
- Costa-Mattioli M, Gobert D, Harding H, Herdy B, Azzi M, Bruno M, Bidinosti M, Ben Mamou C, Marcinkiewicz E, Yoshida M, et al. 2005. Translational control of hippocampal synaptic plasticity and memory by the eIF2 α kinase GCN2. *Nature* **436**: 1166–1173.
- Costa-Mattioli M, Gobert D, Stern E, Gamache K, Colina R, Cuellar C, Sossin W, Kaufman R, Pelletier J, Rosenblum K, et al. 2007. eIF2 α phosphorylation bidirectionally regulates the switch from short- to long-term synaptic plasticity and memory. *Cell* **129**: 195–206.
- Costa-Mattioli M, Sossin WS, Klann E, Sonenberg N. 2009. Translational control of long-lasting synaptic plasticity and memory. *Neuron* **61**: 10–26.
- Delepine M, Nicolino M, Barrett T, Golamaully M, Lathrop GM, Julier C. 2000. EIF2AK3, encoding translation initiation factor 2- α kinase 3, is mutated in patients with Wolcott–Rallison syndrome. *Nat Genet* **25**: 406–409.
- Gallagher SM, Daly CA, Bear MF, Huber KM. 2004. Extracellular signal-regulated protein kinase activation is required for metabotropic glutamate receptor-dependent long-term depression in hippocampal area CA1. *J Neurosci* **24**: 4859–4864.
- Harding HP, Zhang Y, Bertolotti A, Zeng H, Ron D. 2000. PERK is essential for translational regulation and cell survival during the unfolded protein response. *Mol Cell* **5**: 897–904.
- Harding HP, Zeng H, Zhang Y, Jungries R, Chung P, Plesken H, Sabatini DD, Ron D. 2001. Diabetes mellitus and exocrine pancreatic dysfunction in *perK*^{−/−} mice reveals a role for translational control in secretory cell survival. *Mol Cell* **7**: 1153–1163.
- Hou L, Klann E. 2004. Activation of the phosphoinositide 3-kinase-Akt-mammalian target of rapamycin signaling pathway is required for

- metabotropic glutamate receptor-dependent long-term depression. *J Neurosci* **24**: 6352–6361.
- Hou L, Antion MD, Hu D, Spencer CM, Paylor R, Klann E. 2006. Dynamic translational and proteasomal regulation of fragile X mental retardation protein controls mGluR-dependent long-term depression. *Neuron* **51**: 441–454.
- Huber KM, Kayser MS, Bear MF. 2000. Role for rapid dendritic protein synthesis in hippocampal mGluR-dependent long-term depression. *Science* **288**: 1254–1257.
- Huber KM, Gallagher SM, Warren ST, Bear MF. 2002. Altered synaptic plasticity in a mouse model of fragile X mental retardation. *Proc Natl Acad Sci* **99**: 7746–7750.
- Jiang B, Trevino M, Kirkwood A. 2007. Sequential development of long-term potentiation and depression in different layers of the mouse visual cortex. *J Neurosci* **27**: 9648–9652.
- Jiang Z, Belforte JE, Lu Y, Yabe Y, Pickel J, Smith CB, Je HS, Lu B, Nakazawa K. 2010. eIF2 α phosphorylation-dependent translation in CA1 pyramidal cells impairs hippocampal memory consolidation without affecting general translation. *J Neurosci* **30**: 2582–2594.
- Julier C, Nicolino M. 2010. Wolcott–Rallison syndrome. *Orphanet J Rare Dis* **5**: 29.
- Kelleher RJ III, Govindarajan A, Tonegawa S. 2004. Translational regulatory mechanisms in persistent forms of synaptic plasticity. *Neuron* **44**: 59–73.
- Kim TK, Sul JY, Helmfors H, Langel U, Kim J, Eberwine J. 2013. Dendritic glutamate receptor mRNAs show contingent local hotspot-dependent translational dynamics. *Cell Rep* **5**: 114–125.
- Nosyreva ED, Huber KM. 2006. Metabotropic receptor-dependent long-term depression persists in the absence of protein synthesis in the mouse model of fragile X syndrome. *J Neurophysiol* **95**: 3291–3295.
- Reis AF, Kannengiesser C, Jennane F, Manna TD, Cheurfa N, Oudin C, Savoldelli RD, Oliveira C, Grandchamp B, Kok F, et al. 2011. Two novel mutations in the EIF2AK3 gene in children with Wolcott–Rallison syndrome. *Pediatr Diabetes* **12**: 187–191.
- Richter JD, Klann E. 2009. Making synaptic plasticity and memory last: Mechanisms of translational regulation. *Genes Dev* **23**: 1–11.
- Ronesi JA, Huber KM. 2008. Homer interactions are necessary for metabotropic glutamate receptor-induced long-term depression and translational activation. *J Neurosci* **28**: 543–547.
- Rubio-Cabezas O, Patch AM, Minton JA, Flanagan SE, Edghill EL, Hussain K, Balafrej A, Deeb A, Buchanan CR, Jefferson IG, et al. 2009. Wolcott–Rallison syndrome is the most common genetic cause of permanent neonatal diabetes in consanguineous families. *J Clin Endocrinol Metab* **94**: 4162–4170.
- Schmidt EK, Clavarino G, Ceppi M, Pierre P. 2009. SUNSET, a nonradioactive method to monitor protein synthesis. *Nat Methods* **6**: 275–277.
- Seney V, Vattem KM, Delepine M, Rainbow LA, Haton C, Lecoq A, Shaw NJ, Robert JJ, Rooman R, Diatloff-Zito C, et al. 2004. Wolcott–Rallison syndrome: Clinical, genetic, and functional study of EIF2AK3 mutations and suggestion of genetic heterogeneity. *Diabetes* **53**: 1876–1883.
- Sharma A, Hoeffler CA, Takayasu Y, Miyawaki T, McBride SM, Klann E, Zukin RS. 2010. Dysregulation of mTOR signaling in fragile X syndrome. *J Neurosci* **30**: 694–702.
- Shi Y, Vattem KM, Sood R, An J, Liang J, Stramm L, Wek RC. 1998. Identification and characterization of pancreatic eukaryotic initiation factor 2 α -subunit kinase, PEK, involved in translational control. *Mol Cell Biol* **18**: 7499–7509.
- Sonenberg N, Dever TE. 2003. Eukaryotic translation initiation factors and regulators. *Curr Opin Struct Biol* **13**: 56–63.
- Sutton MA, Schuman EM. 2006. Dendritic protein synthesis, synaptic plasticity, and memory. *Cell* **127**: 49–58.
- Takei N, Kawamura M, Hara K, Yonezawa K, Nawa H. 2001. Brain-derived neurotrophic factor enhances neuronal translation by activating multiple initiation processes: Comparison with the effects of insulin. *J Biol Chem* **276**: 42818–42825.
- Thornton CM, Carson DJ, Stewart FJ. 1997. Autopsy findings in the Wolcott–Rallison syndrome. *Pediatr Pathol Lab Med* **17**: 487–496.
- Trinh MA, Kaphzan H, Wek RC, Pierre P, Cavener DR, Klann E. 2012. Brain-specific disruption of the eIF2 α kinase PERK decreases ATF4 expression and impairs behavioral flexibility. *Cell Rep* **1**: 676–688.
- Vattem KM, Wek RC. 2004. Reinitiation involving upstream ORFs regulates ATF4 mRNA translation in mammalian cells. *Proc Natl Acad Sci* **101**: 11269–11274.
- Waung MW, Huber KM. 2009. Protein translation in synaptic plasticity: mGluR-LTD, Fragile X. *Curr Opin Neurobiol* **19**: 319–326.
- Wei J, Sheng X, Feng D, McGrath B, Cavener DR. 2008. PERK is essential for neonatal skeletal development to regulate osteoblast proliferation and differentiation. *J Cell Physiol* **217**: 693–707.
- Zhang P, McGrath B, Li S, Frank A, Zambito F, Reinert J, Gannon M, Ma K, McNaughton K, Cavener DR. 2002. The PERK eukaryotic initiation factor 2 α kinase is required for the development of the skeletal system, postnatal growth, and the function and viability of the pancreas. *Mol Cell Biol* **22**: 3864–3874.
- Zhu PJ, Huang W, Kalikulov D, Yoo JW, Placzek AN, Stoica L, Zhou H, Bell JC, Friedlander MJ, Krnjevic K, et al. 2011. Suppression of PKR promotes network excitability and enhanced cognition by interferon- γ -mediated disinhibition. *Cell* **147**: 1384–1396.

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