Proteasomal Degradation of Eukaryotic Elongation Factor-2 Kinase (EF2K) Is Regulated by cAMP-PKA Signaling and the SCF$^{\text{TRCP}}$ Ubiquitin E3 Ligase*

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**Background:** Eukaryotic elongation factor-2 kinase (EF2K) inhibits the elongation phase of protein translation.

**Results:** EF2K degradation by the ubiquitin-proteasome system (UPS) is regulated by cAMP-PKA signaling and SCF$^{\text{TRCP}}$.

**Conclusion:** Degradation of EF2K enables coordination of UPS function and translational control.

**Significance:** This coordination may be important for achieving proper protein expression to effect cellular adaptations, including synaptic plasticity.

Protein translation and degradation are critical for proper protein homeostasis, yet it remains unclear how these processes are dynamically regulated, or how they may directly balance or synergize with each other. An important translational control mechanism is the Ca$^{2+}$/calmodulin-dependent phosphorylation of eukaryotic elongation factor-2 (eEF-2) by eukaryotic elongation factor-2 kinase (EF2K), which inhibits elongation of nascent polypeptide chains during translation. We previously described a reduction of EF2K activity in PC12 cells treated with NGF or forskolin. Here, we show that both forskolin- and IGF-1-mediated reductions of EF2K activity in PC12 cells are due to decreased EF2K protein levels, and this is attenuated by application of the proteasome inhibitor, MG132. We further demonstrate that proteasome-mediated degradation of EF2K occurs in response to A2A-type adenosine receptor stimulation, and that activation of protein kinase A (PKA) or phospho-mimetic mutation of the previously characterized PKA site, Ser-499, were sufficient to induce EF2K turnover in PC12 cells. A similar EF2K degradation mechanism was observed in primary neurons and HEK cells. Expression of a dominant-negative form of Cul1 in HEK cells demonstrated that EF2K levels are regulated by an SCF-type ubiquitin E3 ligase. Specifically, EF2K binds to the F-box proteins, $\beta$TRCP1 and $\beta$TRCP2, and $\beta$TRCP regulates EF2K levels and polyubiquitylation. We propose that the proteasomal degradation of EF2K provides a mechanistic link between activity-dependent protein synthesis and degradation.

Mounting the proper cellular response to stimulation requires precise temporal and spatial control of protein abundance. Following gene transcription, this precision is achieved largely via translation of mRNAs into proteins and the degradation of existing proteins by the ubiquitin-proteasome system (UPS). Application of either ribosomal or proteasomal inhibitors impairs many types of lasting cellular changes, notably long-term synaptic plasticity and memory (1–4). In the case of long-term potentiation (LTP), concomitant application of both classes of inhibitors rescues the impairments caused by application of either drug alone (5, 6). These findings imply that a balance or coordination of protein synthesis and degradation, rather than engagement of either process per se, may be especially important in control of neuronal plasticity; however the mechanisms by which this coordination is achieved remain to be elucidated.

Eukaryotic elongation factor-2 kinase (EF2K), formerly termed CaMKIII, is a Ca$^{2+}$/calmodulin-dependent protein kinase that inhibits the elongation phase of translation by phosphorylating eukaryotic elongation factor-2 (eEF-2) at Thr-56. Following peptide bond formation, eEF-2 catalyzes the translocation of the ribosome along the mRNA (7, 8), a function in which phospho-eEF-2 is impaired. The Ca$^{2+}$-dependence of EF2K enables it to transduce the activation of various receptors in diverse cell types into changes in protein synthesis with high sensitivity and temporal and spatial specificity (9–12). In addition to its regulation by Ca$^{2+}$, EF2K activity is modulated bidirectionally by phosphorylation by several kinases, including protein kinase A (PKA), AMP-activated kinase (AMPK), p70 S6 kinase, and p90 RSK (13–17). Thus, regulation of EF2K acts to exert translational control downstream of diverse extracellular stimuli and signal transduction pathways.

Previously, we have shown that stimulation of PC12 cells with nerve growth factor (NGF) or the adenyl cyclase-activating drug, forskolin (Fsk), decreases EF2K activity (18, 19). These studies, combined with the observation that EF2K is regulated by the UPS in other cell types (20, 21), led us to hypothesize that this reduction of EF2K activity results from its phosphoryla-

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‡ The abbreviations used are: UPS, ubiquitin-proteasome system; LTP, long-term potentiation; EF2K, eukaryotic elongation factor-2 kinase; BDNF, brain-derived neurotrophic growth factor; eEF, eukaryotic elongation factor; Fsk, forskolin; SCF, Skp1-Cul1-F-box-protein; Chx, cycloheximide.
Degradation of Eukaryotic Elongation Factor-2 Kinase (EF2K)

Degradation of EF2K is a proteasome-dependent process. Here, we show that EF2K is degraded in a proteasome-dependent manner in response to cAMP elevation in PC12 cells, cortical neurons, and HEK cells. This occurs via a mechanism involving activation of PKA, phosphorylation of Ser-499, and recruitment of the ubiquitin E3 ligase, SCECPRCP. These data suggest that cAMP-dependent EF2K degradation serves as a focal point to effect a coordination between UPS function and translational control.

EXPERIMENTAL PROCEDURES

Immunoblotting and Antibodies—Cell lysates were diluted with 5× sample buffer, and samples were run on pre-cast 4–12% polyacrylamide Tris-glycine gradient gels (Invitrogen), except for ubiquitylation assays, wherein 6% gels were used. Gels were transferred to nitrocellulose membranes (Bio-Rad) and blocked with 5% milk in PBS. Membranes were incubated with primary antibodies at 4 °C overnight. All antibodies were diluted in a 1:1 mixture of Odyssey blocking buffer (LI-COR) and PBS with 1% Tween-20. The primary antibodies used were: rabbit anti-EF2K (Angus Nairn, 1:5000), rabbit anti-p–eEF-2 Thr-56 (Cell Signaling, 1:1000), mouse anti-GAPDH (Advanced Immuno Chemicals, 1:10000), mouse anti-FLAG (Sigma, 1:1000), rabbit anti-GFP (AbCam, 1:10,000), mouse anti-GST (Cell Signaling, 1:1000), rabbit anti-p–tyrosine hydroxylase (1:1000, Cell Signaling), mouse anti-p–Akt Ser-473 (Cell Signaling, 1:1000), rabbit anti-Akt (Cell Signaling, 1:1000), mouse anti-p–ERK1/2 Thr-202/Tyr-204 (Cell Signaling, 1:1000), or rabbit anti-ERK1/2 (Cell Signaling, 1:1000). Membranes were then washed three times with PBS-T, incubated for 1 h at 4 °C with IRDye-800-conjugated goat anti-mouse (Rockland, 1:5000) and IRDye-680-conjugated goat anti-rabbit (LI-COR, 1:5000), and washed three more times with PBS-T. Blots were imaged with a Licor Odyssey Infrared Scanner, and quantification was performed using Licor Odyssey software. When necessary, membranes were stripped using a solution containing 25 mM glycine and 2% SDS, pH 2.0, at 55 °C. Upon confirmation that the signal had been removed, stripped membranes were re-blocked, and re-probed with primary and secondary antibodies. In all cases, data shown for EF2K levels were normalized to GAPDH levels measured in the same sample.

Cell Culture—For PC12 cells (ATCC), 6- or 12-well plates were coated with a solution of 0.05 mg/ml of collagen-I from rat tail (Sigma) in 0.02 N acetic acid for at least 1 h and washed with PBS prior to plating. Cells were grown in RPMI 1640, supplemented with 10% horse serum and 5% FBS (all from Invitrogen). HEK 293-T cells (ATCC) were grown on un-coated plates in DMEM (Invitrogen), supplemented with 10% FBS.

Stimulation of PC12 Cells or HEK Cells—PC12 cells were transferred to serum-free media 1 h prior to the addition of any reagents. MG132 (Tocris, 25 μM in DMSO) was added 30 min prior to the start of stimulation. Cells were stimulated with either forskolin (Tocris, 10 μM in DMSO), IGF-1 (Millipore, 100 ng/ml in water), CGS 21680 hydrochloride (Tocris, 100 nM in DMSO), or 6-BNZ-cAMP (Sigma, 100 μM in water) for up to 48 h and lysed in buffer containing 1% Nonidet P-40, 50 mM Tris, pH 7.4, 200 mM NaCl, 1 mM EDTA, and protease and phosphatase inhibitor cocktails (Sigma, 1:100). For protein turnover experiments, cells were treated with cycloheximide (Chx, Sigma, 30 μg/ml in DMSO) in serum-containing media and lysed at the indicated times. Stimulation experiments in HEK cells were performed 48 h post-transfection. Cells were transferred to media containing serum and cycloheximide (30 μg/ml) in the presence or absence of forskolin (10 or 50 μM) and lysed at the indicated times.

Culture and Stimulation of Primary Cortical Neurons—Cortical cultures were prepared from rat embryos according to standard protocols. All procedures were approved by the Yale Animal Care and Use Committee (YACUC) and followed the NIH Guide for the Care and Use of Animals. Pregnant rats (E18) were euthanized with CO2 asphyxiation. Embryos were isolated and brains dissected and bathed in Hank’s Balanced Salt Solution (HBSS) containing 1% penicillin-streptomycin (both from Invitrogen). Cortices were isolated and digested at 37 °C in papain (Worthington), diluted 1:500 in the dissection solution, for 1 h, during which time the solution was twice tritiated 20–30 times. The digested tissue was collected by centrifugation (5 min at 100 × g), the papain solution was removed, and the cells were resuspended in plating media consisting of Neurobasal supplemented with B27, Glutamax, sodium pyruvate, 1 mM HEPES, 1% penicillin-streptomycin, and 10% FBS (all from Invitrogen). Cells were counted in Trypan Blue using a hemocytometer, and 0.7 × 10⁶ cells were plated per well on poly-L-lysine-coated 6-well plates. On the following day, the media was replaced with Neurobasal containing all of above supplements except FBS. Neurons were maintained in this media and experiments were performed after 14–15 days in vitro. Forskolin (10 μM) and BDNF (Millipore, 100 ng/ml in water) were added to the conditioned media for 3 or 6 h, and cells were lysed as above. MG132 (25 μM) was added 30 min prior to the start of stimulation.

Plasmids and Transfection—GST-F-box, FLAG-DN-Cull, and shβTRCP constructs were generous gifts from J. W. Harper. The GFP-EF2K construct was generated by sub-cloning rat EF2K into pEGFP-N1 (Clontech) and point mutants were generated using standard PCR procedures and validated by DNA sequencing. For transfections, DNA was incubated with Lipofectamine 2000 (Invitrogen) in Opti-MEM media (Invitrogen) and added to cells. Protein expression was examined 24–48 h later.

GST Pull-downs—HEK cells were transfected with GFP-EF2K and GST-tagged F-box constructs and lysed in Nonidet P-40 buffer as above. Lysates were cleared by centrifugation (14,000 rpm for 20 min), and protein concentration was assayed by BCA (Pierce). For each pull-down, 2 mg of protein was used, diluted in 1 ml of lysis buffer, and 50 μl of glutathione-Sepharose beads (50% slurry, GE Healthcare) were added to the lysates, which were rotated for 30 min at 4 °C. The beads were washed three times with lysis buffer, and bound proteins were eluted by boiling in 100 μl of 2× SDS-PAGE sample buffer.

EF2K Ubiquitylation Experiments—PC12 cells were transfected with GFP-EF2K, with or without GST-βTRCP-1 and treated with MG132 (25 μM) for 2 h to accumulate polyubiquitylated proteins. Lysates were boiled in 1% SDS for 10 min to both inactivate de-ubiquitylating enzymes and dissociate any ubiquitylated contaminates that could otherwise co-immuno-
precipitate with EF2K. The lysates were diluted to 0.1% SDS prior to the addition of monoclonal GFP antibodies (19C8 and 19F7 from Memorial Sloan-Kettering), and were rotated overnight at 4 °C. The beads were washed once each in a series of stringent buffers: 1) 10 mM Tris, pH 8.0, 500 mM NaCl, 0.5% Nonidet P-40, 0.05% SDS; 2) 10 mM Tris, pH 8.0, 150 mM NaCl, 0.5% Nonidet P-40, 0.05% SDS, 0.5% deoxycholate; 3) 10 mM Tris, pH 8.0, 0.05% SDS (22). Bound proteins were eluted by boiling in 2× SDS-PAGE sample buffer.

Statistics—Data were analyzed by one-sample t test, one-way ANOVA with Tukey’s post-hoc tests, or two-way ANOVA with Bonferroni or Tukey’s post-hoc tests, as appropriate. Significance was considered to be p < 0.05. All data are representative of at least three independent experiments. All graphs are displayed with error bars representing the standard error of the mean.

RESULTS

Proteasome-dependent Degradation of EF2K during Treatment of PC12 Cells with Forskolin or IGF-1—To test whether the reduced EF2K activity that we previously observed during cAMP elevation in PC12 cells is caused by proteasomal degradation of EF2K, we treated PC12 cells for 6 h with forskolin (Fsk, 10 μM) in the absence or presence of the proteasome inhibitor, MG132 (25 μM). Fsk significantly reduced EF2K protein levels relative to control (49.38 ± 6.17, n = 7), and caused a corresponding decrease in eEF-2 phosphorylation at Thr-56 (Fig. 1, A and B). Building on our earlier results with NGF, we found that IGF-1 treatment (100 ng/ml) for 6 h also reduced EF2K protein levels (62.46 ± 7.24, n = 4) and eEF-2 phosphorylation (Fig. 1, A and B). Both the Fsk- and IGF-1-mediated decreases in EF2K levels were blocked by co-application of MG132 (Fsk+MG132: 84.53 ± 6.187, n = 5; IGF-1+MG132: 93.47 ± 4.35, n = 2), indicating they are proteasome-dependent. Interestingly, while co-application of Fsk and MG132 restored p-eEF-2 to control levels, co-application of IGF-1 and MG132 decreased p-eEF-2 relative to control samples, which may be caused by the accumulation of EF2K that has been inhibited by phosphorylation by p70 S6 kinase (16).

To confirm that Fsk causes degradation of EF2K per se, rather than activating a proteasome-dependent process that decreases EF2K levels by another means (e.g. reduced transcription or translation), we performed a chase experiment in PC12 cells to selectively examine the effect of Fsk on EF2K stability in the presence of the protein synthesis inhibitor, cycloheximide. We found that Fsk stimulation significantly hastened EF2K degradation (Fig. 1, C and D), shortening its half-life from 4.3 h to 2.0 h. Together, these data indicate that EF2K itself is targeted for proteasomal degradation in response to Fsk or IGF-1 stimulation.

We examined whether a more physiological means of increasing cAMP—activation of the Gs-coupled adenosine A2A receptor also induced EF2K degradation. PC12 cells were treated for 3 or 6 h with the A2A receptor agonist, CGS 21680 (100 nM), in the presence or absence of MG132. CGS 21680 application for either 3 or 6 h significantly reduced EF2K levels relative to control (CGS 3 h: 65.95 ± 12.02, n = 3; CGS 6 h: 49.31 ± 12.02, n = 3) and concomitantly increased PKA activity, as assessed by phosphorylation of TH at Ser-40 (Fig. 2, A and B). The addition of MG132 significantly blocked the CGS 21680-mediated decrease in EF2K levels at both time points.
Degradation of Eukaryotic Elongation Factor-2 Kinase (EF2K)

EF2K Degradation Is Induced by cAMP/Growth Factor Signaling in Neurons and HEK Cells—To assess whether the degradation of EF2K we described in PC12 cells represents a more general translational control mechanism, we investigated whether similar phenomena may be engaged in neurons and HEK cells. Primary cortical neurons (DIV 14–15) were treated with Fsk (10 \( \mu \)M) and BDNF (100 ng/ml) in the absence or presence of MG132. In contrast to our findings in PC12 cells, treatment with either Fsk or BDNF alone for 6 h was not sufficient to change EF2K levels in neurons (data not shown), but co-application of Fsk and BDNF significantly reduced EF2K levels after 3 or 6 h (3 h: 86.00 \( \pm \) 3.39, \( n = 5 \); 6 h: 72.23 \( \pm \) 2.548, \( n = 7 \)) (Fig. 3, A and B). This decrease in EF2K levels was accompanied by a decrease in p-eEF-2 (data not shown). As in PC12 cells, the effect of co-application of Fsk and BDNF was attenuated by administration of MG132 at both 3 and 6 h time points (3 h + MG132: 99.26 \( \pm \) 0.67, \( n = 4 \); 6 h + MG132: 86.79 \( \pm \) 3.48, \( n = 4 \)) (Fig. 3, A and B), indicating that the effect is proteasome-dependent.

In HEK cells, the level of endogenous EF2K is low compared with PC12 cells, and therefore we transiently transfected GFP-EF2K and examined the effect of Fsk (10 or 50 \( \mu \)M) treatment on its stability during a 48 h chase with Chx (30 \( \mu \)g/ml). As in PC12 cells, application of Fsk dose-dependently accelerated GFP-EF2K turnover in HEK cells (Fig. 4, A and B), shortening its half-life from 24.8 h under control conditions to 9.4 and 5.9 h for the 10 \( \mu \)M and 50 \( \mu \)M doses, respectively, during the initial 24 h stimulation period.

Mechanism of cAMP-dependent Regulation of EF2K Turnover—We hypothesized that activation of PKA mediates the effects of Fsk and A2A receptor activation because EF2K is known to be a PKA substrate (13–15), and we previously observed that a mutant strain of PC12 cells (A126–1B2) that is known to be a PKA substrate (13–15), and we previously observed that a mutant strain of PC12 cells (A126–1B2) that is deficient in PKA does not exhibit reduced EF2K activity during Fsk treatment (19). Treatment of PC12 cells with the selective PKA activator, 6-BNZ cAMP (100 \( \mu \)M) was found to be sufficient to significantly decrease EF2K levels relative to control (68.84 \( \pm \) 6.35, \( n = 3 \)) after 1 h (Fig. 5, A and B). EF2K levels returned to baseline after continued stimulation with 6-BNZ cAMP for up to 6 h. Activation of a parallel cAMP-dependent signaling module, exchange protein activated by cAMP (Epac), with 8-CPT cAMP (100 \( \mu \)M) did not affect EF2K levels in PC12 cells (data not shown).

We next investigated whether PKA phosphorylation of EF2K affected its degradation. Two EF2K residues, Ser-365 and Ser-499, have previously been identified as PKA phosphorylation sites (15), and we generated GFP-tagged EF2K constructs bearing mutations of either of these sites to putatively phosphomimetic residues (365D and 499E) and examined the stability of these mutants in PC12 cells during a cycloheximide chase (Fig. 5, C and D). The 499E mutant form of EF2K, but not 365D, exhibited lower steady-state expression and accelerated turnover relative to wild-type GFP-EF2K. Together, these data suggest that cAMP-PKA signaling accelerates EF2K degradation via phosphorylation of Ser-499.
**EF2K Is a Substrate of SCF<sup>βTRCP</sup>**—To further elucidate the mechanism by which phosphorylated EF2K is targeted to the proteasome, we examined whether phosphorylated EF2K is selectively polyubiquitylated by a Skp1-Cul1-F-box-protein (SCF)-type ubiquitin E3 ligase. SCF complexes comprise the scaffolding protein, Cul1, which recruits an E2 ubiquitin-conjugating enzyme via its interaction with the RING-finger protein, Rbx1, and substrate proteins via its interaction with the adaptor protein, Skp1, which in turn binds to an F-box protein, which serves as the substrate receptor. F-box proteins give rise to the selectivity of SCF-type ligases, in many cases binding to substrates in a manner dependent on phosphorylation of a specific motif, termed a phospho-degron (23, 24).

To evaluate the potential role of SCF complexes in regulating EF2K levels, we co-transfected HEK cells with GFP-EF2K in the presence or absence of a FLAG-tagged dominant-negative truncation mutant of Cul1 (amino acids 1–452) that abrogates its binding to Rbx1, and thus to the machinery for substrate ubiquitylation (25, 26). Co-expression of DN-Cul1 increased GFP-EF2K levels nearly 10-fold (982/110 062 229, n = 4) relative to cells expressing GFP-EF2K alone (Fig. 6, A and B). These data strongly suggest that a SCF-type E3 ubiquitin ligase negatively regulates EF2K stability.

We next sought to identify the F-box protein that recruits EF2K to the SCF complex. HEK cells were co-transfected with GFP-EF2K and one of a panel of GST-tagged F-box proteins (26, 27). Lysates were incubated with glutathione beads to pull-down GST-F-box proteins and eluates were immunoblotted for GFP-EF2K to assess binding. GFP-EF2K was found to bind to TRCP1 and TRCP2, but not to any of the other F-box pro-
Degradation of Eukaryotic Elongation Factor-2 Kinase (EF2K)

A Input

GFP (EF2K)
GST (Fbox)
GAPDH

GST Pull-down

GFP (EF2K)
GST (Fbox)

B Input

+GST-βTRCP-1

GFP-EF2K
GST-βTRCP-1
GAPDH

GST Pulldown

FIGURE 7. EF2K binds to βTRCP1 and βTRCP2. A, HEK cells were co-transfected with GFP-EF2K and one of a panel of GST-tagged F-box proteins. Cell lysates were incubated with glutathione beads, and binding was assessed in inputs (upper panel) and eluates (lower panel) by performing SDS-PAGE and immunoblot for GFP and GST. * indicates GST-Fbw7a, which migrates at a similar but distinct molecular weight as GFP-EF2K by SDS-PAGE. B, HEK cells were co-transfected with GST-βTRCP1, and either wild-type GFP-EF2K, or S440/S444 AA, or Y442F mutant forms and pull-downs were performed as above. Binding was assessed in inputs (upper panel) and eluates (lower panel) by performing SDS-PAGE and immunoblot for GFP and GST.

teins tested (Fig. 7A). While EF2K contains a consensus sequence for the phospho-degron domain recognized by the βTRCP proteins (28), the sequence DSGYPS containing Ser-440 and Ser-444, neither mutation of both Ser residues nor of Tyr-442 appeared sufficient to disrupt EF2K binding to βTRCP-1 (Fig. 7B).

To determine whether βTRCP regulates EF2K expression, HEK cells were co-transfected with GFP-EF2K and a previously characterized shRNA that targets both βTRCP1 and βTRCP2 (26). Knock-down of βTRCP caused a >14-fold increase (1441 ± 276; n = 3) in steady-state EF2K levels relative to cells transfected with GFP-EF2K alone (Fig. 8, A and B).

We also investigated whether co-transfection with sh-βTRCP or DN-Cul1 affected Fsk-mediated turnover of GFP-EF2K during a cycloheximide chase. As in our earlier experiments, both sh-βTRCP and DN-Cul1 significantly increased the steady-state expression of GFP-EF2K and Fsk treatment significantly hastened GFP-EF2K degradation (Fig. 9, A and B). After 8 h treatment with Chx and Fsk, levels of GFP-EF2K normalized to the control baseline were higher in both sh-βTRCP (124% ± 15) and DN-Cul1 (115% ± 13) transfected cells than control-transfected cells (59% ± 5), suggesting that SCFβTRCP is involved in both steady-state and Fsk-stimulated EF2K turnover in HEK cells.

Finally, we examined whether βTRCP1 regulated EF2K ubiquitination in PC12 cells. We expressed GFP-EF2K in PC12 cells in the absence or presence of GST-βTRCP1 expression and treated cells with MG132 for 2 h to allow the accumulation of polyubiquitylated proteins. GFP-EF2K was then immunoprecipitated from lysates, and its ubiquitylation status was assessed by immunoblot (Fig. 10). Expression of βTRCP1 increased the polyubiquitylation of EF2K, indicated both by the accumulation of heavy molecular weight species of EF2K and by an increase in the ubiquitin signal in immunoprecipitates.

DISCUSSION

Our data demonstrate that EF2K is degraded by the proteasome in response to cAMP signaling in PC12 cells, neurons, and HEK cells. Adenosine A2A receptor or PKA activation and phosphorylation of EF2K on Ser-499 appear to be sufficient to engage this degradation, presumably by recruiting the ubiquitin
Degradation of Eukaryotic Elongation Factor-2 Kinase (EF2K)

EF2K activity is known to be regulated by increases in intracellular [Ca^{2+}], which occurs within seconds, and phosphorylation, which occurs within minutes. Here, we demonstrate that degradation by the UPS represents an additional mode of regulation of EF2K, one which occurs over the course of a few hours. These modes of regulation are not mutually exclusive and likely interact. For example, we observed that while eEF-2 phosphorylation levels in Fsk-stimulated cells treated with MG132 were similar to those of control cells, in IGF-1-stimulated cells, MG132 treatment led to a decrease of p-eEF-2, which may reflect an accumulation of EF2K bearing inhibitory phosphorylation downstream of p70 S6 kinase (16).

Together, our data suggest an cAMP-dependent mechanism by which receptor activation can engage both the ubiquitin-proteasome system and regulation of protein translation, which in turn may enable protein synthesis and degradation to be balanced or coordinated to effect cellular changes. Such a coordination appears to be necessary for long-term synaptic plasticity based on studies showing that simultaneous inhibition of protein synthesis and UPS-mediated degradation rescues deficits in LTP caused by inhibition of either alone (5, 6). A balance between protein synthesis and degradation may also play a role in homeostatic synaptic plasticity, as inhibiting and driving action potential firing in cultured neurons produces reciprocal changes in synaptic protein levels that are mediated in part by the UPS (29). Interestingly, regulation of EF2K activity has been shown to play a role in both these types of plasticity (10, 30), but it remains to be determined whether proteasomal degradation of EF2K is involved in some way in long-term forms of plasticity.

Activation of EF2K has been shown not only to decrease overall protein synthesis by inhibiting eEF-2, but to paradoxi-
cally activate the translation of some messages. For example, NMDA receptor stimulation in synaptic preparations resulted in rapid increases in p-eEF-2 concomitant with decreased overall protein synthesis, and yet an increase in the translation of α-CaMKII (31). This form of EF2K-mediated translational control appears to be required for hippocampal metabolic glutamate receptor-mediated long-term depression (mGluR-LTD), in this case by activating the translation of Arc, as this form of plasticity is abolished in EF2K knock-out mice (30). Thus, degradation of EF2K by the UPS is expected to not only facilitate protein synthesis overall, but to decrease the translation of such anomalously regulated transcripts, thereby remodeling the repertoire of actively translating messages and contributing to long-term cellular changes.

Our findings build on a growing body of literature describing UPS-mediated degradation of proteins involved in translational control. In particular, SCF\(\beta\)-TRCP has been shown to mediate the phosphorylation-dependent degradation of several such proteins, including the eIF4A inhibitor, PDCD4 (32), the mTOR inhibitor, DEPTOR (33–35), and the RNA-binding protein, CPEB (36). Additionally, in neuronal systems, plasticity-inducing stimulation has been shown to cause the degradation of the RNA-induced silencing complex (RISC) protein, Armitage/MOV10 (37, 38), and the RNA-binding protein, FMRP (39). It is striking that, as is the case with EF2K, all of the examples above involve the degradation of a negative regulator of protein synthesis, suggesting that engagement of the UPS may play a general role in activating translation.

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