Translational Control of C-terminal Src Kinase (Csk) Expression by PRL3 Phosphatase*

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Phosphatase of regenerating liver 3 (PRL3) is up-regulated in cancer metastases. However, little is known of PRL3-mediated cellular signaling pathways. We previously reported that elevated PRL3 expression increases Src kinase activity, which likely contributes to the increased tumorigenesis and metastasis potential of PRL3. PRL3-induced Src activation is proposed to be indirect through down-regulation of Csk, a negative regulator of Src. Given the importance of PRL3 in tumor metastasis and the role of Csk in controlling Src activity, we addressed the mechanism by which PRL3 mediates Csk down-regulation. PRL3 is shown to exert a negative effect on Csk protein synthesis, rather than regulation of Csk mRNA levels or protein turnover. Interestingly, the preferential decrease in Csk protein synthesis is a consequence of increased eIF2 phosphorylation resulting from PRL3 expression. Reduced Csk synthesis also occurs in response to cellular stress that induces eIF2 phosphorylation, indicating that this regulatory mechanism may occur in response to a wider spectrum of cellular conditions known to direct translational control. Thus, we have uncovered a previously uncharacterized role for PRL3 in the gene-specific translational control of Csk expression.

The PRL (phosphatase of regenerating liver)2 phosphatases represent a novel class of protein-tyrosine phosphatases (PTPs) that are prenylated at their C terminus. PRL1 was originally identified as an immediate early gene in regenerating liver (1). Subsequently, the PRL phosphatases have been implicated in the control of a number of cell growth and migratory processes. In particular, PRL3 has been recognized as a prognostic marker and a therapeutic target for metastatic tumors (2, 3). PRL3 is overexpressed in metastatic lesions derived from colorectal cancers, while its expression is undetectable in primary tumors and normal colorectal epithelium (3, 4). Elevated PRL3 expression has also been reported in other highly metastatic cancers including gastric (5), Hodgkin’s lymphoma (6), liver carcinoma (7), breast (8), ovarian (9), and melanoma (7). In addition, cells overexpressing PRL3 can induce metastatic tumor formation in mice (10), whereas knockdown of endogenous PRL3 in cancerous cells using small interfering RNA abrogates cell motility and the ability to metastasize both in vitro and in vivo (11–13). Collectively, these studies show that an excess of PRL3 is a key contributing factor to the acquisition of metastatic properties of tumor cells.

Although considerable evidence has now accumulated implicating a causal role for PRL3 in tumor metastases, little is known about PRL3-mediated cellular signaling pathways. We recently found that up-regulation of PRL3 activates the Src kinase, which initiates a number of signal pathways responsible for increased cell growth and motility (14). This is consistent with Src being activated in a large number of malignancies and cancer metastases (15–17). Src activity is controlled by autophosphorylation of Tyr-416 in the kinase domain and through phosphorylation of Tyr-527 in the C-terminal tail. The latter phosphorylation event, catalyzed by the C-terminal Src kinase (Csk) (18), represses Src activity through an inhibitory intramolecular pTyr-527-SH2 interaction (19, 20). We showed that the PRL3-induced Src activation results from a decrease in the inhibitory pTyr-527 level (14). Interestingly, PRL3 does not catalyze pTyr-527 dephosphorylation. Rather, the observed decrease in pTyr-527 is caused by a reduction in the Csk protein. Thus, the mechanism for Src activation in PRL3 cells is through down-regulation of Csk. Importantly, this mechanism may also be operative in colon cancer cell lines, as reduced Csk has been found to correlate inversely with elevated Src activity in highly metastatic colon and hepatocellular carcinoma (21–23), whereas overexpression of Csk reduces Src activity and suppresses tumor metastasis in vivo (24).

To gain further insight into the PRL3-mediated disease processes, it is important to define the mechanism by which PRL3 down-regulates Csk. Csk is a nonreceptor tyrosine kinase that shares much homology with the Src family kinases. Csk functions to suppress Src activity by phosphorylating its C-terminal Tyr-527. This modification appears to have profound biological significance, as mice lacking Csk die in utero at week 9, and cells harvested from the embryos exhibit unusually high Src activity (25). Despite the fundamental role of Csk in controlling Src activity, there is very limited understanding of how Csk is regulated. Unlike most tyrosine kinases, the activity of Csk is not modulated by phosphorylation (18). The only known mode of Csk regulation is through binding to Cbp (Csk-binding protein), which localizes Csk to the membrane (26). In the current study, we have examined the effect of PRL3 on Csk expression,
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both at the message and protein levels. Our results suggest that elevated PRL3 expression leads to a reduction in Csk protein synthesis, by a mechanism involving the eIF2 kinase pathway. This represents the first example in which up-regulation of a PTP generates a gene-specific down-regulation of a tyrosine kinase through the general protein translation machinery.

EXPERIMENTAL PROCEDURES

Materials—Anti-Csk antibodies were from BD Biosciences. Anti-p21 antibody was from Santa Cruz Biotechnology. Anti-eIF2 and phospho-eIF2 (Ser-51) antibodies were purchased from Cell Signaling. Cycloheximide, rapamycin, thapsigargin, MG132, metilmamine, and chloroquine were purchased from Sigma. GADD34 constructs were generous gifts from Dr. David Ron (New York University School of Medicine).

Cell Culture and Transfection—HEK293 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (Invitrogen), penicillin (50 units/ml), and streptomycin (50 μg/ml) under a humidified atmosphere containing 5% CO₂. SW480 and SW48 colorectal cancer cell lines were cultured in Leibovitz's L-15 medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin; the SW620 colorectal cancer cell line was maintained in DMEM supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. Human PRL3 was subcloned into the pcDNA3 expression vector. HEK293 cells were seeded at 40% confluence in antibiotic-free medium and grown overnight. Transfection was performed using Lipofectamine (Invitrogen) according to the manufacturer's recommendations. Twenty-four hours after transfection, 0.5 mg/ml G418 was added to the culture medium. Stable clones were picked after 2 weeks of selection. To study the effect of GADD34 on eIF2 phosphorylation, pcDNA4-GADD34 was used to stably transfect HEK293-PRL3 cells. Stable clones were picked after selection with 400 μg/ml Zeocin for 2 weeks.

mRNA Extraction and RT-PCR—mRNA from the cells was prepared using Trizol reagent (Invitrogen). mRNA was treated by DNase and quantified by absorbance at 260 and 280 nm. RT-PCR was performed using the Invitrogen SuperScript one-step RT-PCR kit. Reverse transcription was done at 50 °C for 30 min, and cDNA was amplified for 30 or 32 cycles (94 °C, 30 s; 55 °C, 1 min; 72 °C, 20 s). The sequences of specific primers were as follows: Csk sense, 5’-CATCCGAGCCCAA-CTACGTCAGG-3’; and antisense, 5’-GAAAGGTCTCAA-GATGGATGC-3'; 18S ribosome sense, 5’-GCACGCTAGG-AGGTGAAATTG-3’; and antisense, 5’-TTGGCATAATGC-TTTGCGTC-3’; β-actin sense, 5’-GGTACCACACTGTC-CCAGG-3’, and antisense, 5’-ACATCTGCTGGAAATGTT-GAC-3’. The PCR products were separated in a 1% agarose gel and visualized by staining with ethidium bromide.

Real-time Quantitative RT-PCR Analysis—The Csk mRNA level was determined by a two-step quantitative RT-PCR protocol using the fluorescent intercalating dye SYBR-Green RT-PCR kit (Invitrogen) and an ABI Prism 7700 sequence detection system (Applied Biosystems). 250 ng of RNA were used for first strand cDNA synthesis. Primer sequences for Csk and 18S were the same as those used for the RT-PCR experiments. 18S rRNA was used as an internal control. The cycle threshold (Ct) value, defined as the PCR cycle at which a statistically significant increase of reporter fluorescence is first detected, was used as a measure for the starting copy numbers of target genes. The ratio of the Cₜ value of Csk mRNA to that of 18S in each cell line was determined as the relative Csk mRNA level.

Immunoblotting and Immunoprecipitation—Cells were grown to 70% confluency, washed with ice-cold phosphate-buffered saline, and lysed on ice for 30 min in 1 ml of lysis buffer (50 mM Tris-HCl, pH 7.5, 5 mM EGTA, 150 mM NaCl, 10 mM sodium phosphate, 10 mM sodium fluoride, 1 mM sodium pervanadate, 1 mM benzamidine, 1% Triton X-100, 10 μg/ml leupeptin, and 5 μg/ml aprotinin). Cell lysates were cleared by centrifugation at 15,000 rpm for 15 min. The lysate protein concentration was estimated using the BCA protein assay kit (Pierce). For immunoprecipitation, 10 μg of antibody was added to 1 ml of cell lysate and incubated at 4 °C for 2 h. 20 μl of protein A/G-agarose beads were then added and incubated for another 2 h. After extensive washing, the protein complex was boiled with sample buffer, separated by SDS-PAGE, transferred electrophoretically to nitrocellulose membrane, and immunoblotted with appropriate antibodies followed by incubation with horseradish peroxidase-conjugated secondary antibodies. The blots were developed by the enhanced chemiluminescence technique (ECL kit, Amersham Biosciences). Band intensities were analyzed with the software ImageJ developed by NIH. Data shown represent the results of at least three independent experiments.

Pulse Chase Experiment—Cells were grown to 70% confluency, then treated with methionine- and cysteine-free DMEM supplemented with dialyzed 10% fetal bovine serum for 30 min. Cells were then metabolically labeled with 250 μCi/ml [35S]Met/Cys for 4 h. After washing with PBS, cells were cultured in DMEM, supplemented with 10% fetal bovine serum, 500 mg/ml cysteine, and 100 mg/ml methionine for 0, 4, or 24 h. Cells were collected and lysed, and Csk was immunoprecipitated from the cell lysate with Csk antibody and protein A-Sepharose. Proteins in the immunocomplex were separated by SDS-PAGE, and the newly synthesized Csk was visualized by autoradiography. The overall rate of protein synthesis was determined by measuring the amount of radioactivity associated with the total protein collected by precipitation with 10% trichloroacetic acid.

Polyribosome Isolation and Northern Blot Analysis—Cells were grown to 70% confluency in one 10-cm dish. Cells were washed in ice-cold phosphate-buffered saline three times, and lysed in 0.3 ml of lysis buffer (20 mM Tris-HCl, pH 7.5, 100 mM KCl, 10 mM MgCl₂, 0.5% Nonidet P-40). Cell preparations were then passed through a 25-gauge needle five times. Nuclei and debris were removed by centrifugation of the cell lysate in a microcentrifuge at 10,000 rpm for 10 min. The cleared lysate was then placed onto the top of a 15% sucrose gradient, which was prepared in lysis buffer without Nonidet P-40. Seventeen fractions (~0.7 ml/fraction) were collected by monitoring the A₂₅₄ absorbance using a spectrophotometer after centrifugation at 40,000 rpm in Beckman SW41 for 2 h. RNA from different fractions were prepared using the Invitrogen Trizol reagent. RNA samples were suspended in 6× loading buffer and denatured at 75 °C for 5 min, and the preparations were
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PRL3 Has No Effect on Csk Protein Stability—The level of cellular proteins is the net result of protein synthesis and degradation. To explore the molecular basis for the PRL3-induced Csk down-regulation, we first examined whether Csk is ubiquitinated and whether Csk is being actively degraded by various cellular proteases. Cells were treated with small pharmacological reagents MG132, methylamine, or chloroquine to test if there is an increase in Csk protein. MG132 is a potent inhibitor of the 26S proteasome (27), while methylamine and chloroquine are lysosomotropic agents, which enter the lysosome and neutralize the H+ ion gradient, leading to the inhibition of lysosomal proteases that function optimally at acidic pH (28–29). As shown in Fig. 2, the Csk protein level in PRL3 cells is substantially lower (3.5-fold) than that in control cells, which is in agreement with our earlier observations (14). It appears that Csk is not ubiquitinated as judged by the lack of higher molecular weight species after the proteasome inhibitor treatment. Moreover, Csk is not subject to active degradation because there is no measurable change in the Csk protein level after the cells are exposed to proteasome and lysosomal protease inhibitors.

To further examine whether the lower Csk level in PRL3-expressing cells is due to altered Csk protein stability, we measured the Csk protein levels after the cells were treated with cycloheximide (CMX), which blocks protein synthesis. Cell lysates were resolved by SDS-PAGE and subjected to Western blotting with anti-Csk antibodies. As can be seen in Fig. 3A, no change in Csk protein was observed even after 24 h of CHX treatment in both PRL3 and vector control cells. As an experimental control, we also determined the cyclin-dependent kinases (CDKs) inhibitor p21 level before and after the cells were incubated with CHX. p21 is an unstable cell cycle regulator with a very short half-life (30, 31). As expected, there was a significant decrease in p21 levels after the cells were treated for as short as 0.6 h with CHX (Fig. 3B), which is in agreement with previous studies (30). Collectively, our results indicate that Csk is a long-lived protein and that the decreased Csk level in PRL3 cells is not due to increased protein degradation.

Up-regulation of PRL3 Inhibits Csk Protein Synthesis—Given that there was no indication that Csk is regulated at the level of protein turnover, we hypothesized that the decreased Csk protein levels in PRL3 cells could be caused by a lower rate of

RESULTS

PRL3 Does Not Affect Csk mRNA Levels—We previously established several stable HEK293 cell lines in which the levels of ectopically expressed PRL3 were 3–5-fold higher than that of the endogenous PRL3 in vector control cells (14). We discovered that elevated PRL3 expression causes a reduction in Csk protein, leading to Src activation. Csk down-regulation, in theory, could occur at the levels of either mRNA or protein. For example, a reduction in Csk may result from a decrease in gene transcription, an increase in mRNA decay, a decrease in protein synthesis, or an increase in protein turnover. To determine whether Csk expression is influenced by PRL3, we performed RT-PCR experiments to measure the Csk mRNA level (Fig. 1A).

No differences in Csk mRNA were observed between the control and PRL3 cell lines. To further confirm the RT-PCR results, we next measured Csk mRNA using quantitative real time RT-PCR. All real time RT-PCR reactions were done in triplicate, and the C\textsubscript{T} (threshold cycle) value of Csk was determined and normalized against those of the 18S rRNA. Again, Csk mRNA levels were similar between the two cell lines (Fig. 1B). These results indicate that down-regulation of Csk by PRL3 does not change the RNA level, suggesting a role for post-transcriptional mechanisms.
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**A**

|          | Vector | PRL3 |
|----------|--------|------|
| CHX (hr) | 0      | 13   |
|          | 0      | 13   |
|          | 24     | 24   |
| Csk      |        |      |
| β-Actin  |        |      |

**B**

|          | Vector | PRL3 |
|----------|--------|------|
| CHX (hr) | 0      | 0.3  |
|          | 0.3    | 0.6  |
|          | 0.6    | 2    |
|          | 2      | 4    |
|          | 4      | 8    |
| p21      |        |      |
| β-Actin  |        |      |

**FIGURE 3.** PRL3 has no effect on Csk protein turnover. PRL3 and vector control cells were treated with 20 μg/ml cycloheximide for the indicated amounts of time. Csk, β-actin, and p21 protein levels were measured by immunoblotting with specific antibodies.

Translation relative to the control cell line. To test this hypothesis and further confirm that Csk protein turnover is not altered by PRL3 expression, we conducted radiolabeled pulse-chase experiments in PRL3 cells and vector control cells. The cells were cultured in a medium containing exogenously added [35S]methionine and [35S]cysteine to allow for metabolic labeling of the newly synthesized proteins for 4 h. This was followed by a chase in the presence of cold methionine/cysteine for various periods of time. Cells were then lysed, and Csk protein from the cell lysates was immunoprecipitated with anti-Csk antibodies and analyzed by SDS-PAGE. The newly synthesized Csk was determined by autoradiography. Total Csk and β-actin protein levels in the cell lysate were measured by immunoblotting with specific antibodies. The results showed that the newly synthesized Csk in PRL3 cells was 3.5-fold lower than that of the control cells (Fig. 4A), although the overall rate of [35S] incorporation into proteins in PRL3 cells was only 20% less than the control cell line (Fig. 4B). This decrease in Csk protein synthesis mirrored the decrease in steady-state levels of Csk protein levels as judged by the immunoblot (Fig. 4A). Meanwhile, the levels of β-actin remained unchanged when comparing the control and PRL3 cells (Fig. 4A). Furthermore, the newly synthesized Csk protein was stable within 24 h of chase in both control and PRL3 cells (Fig. 4A), which was fully consistent with the cycloheximide and protease inhibitor studies, indicating that Csk is a long-lived protein (Figs. 2 and 3). Taken together, these results suggest that PRL3 preferentially decreases the rate of Csk protein synthesis.

To further establish that the PRL3-mediated Csk down-regulation occurs at the translational level, we examined the effect of PRL3 on Csk mRNA association with ribosomes. Actively translating polyribosomes can be separated from ribosomal subunits and monoribosomes using sucrose gradient ultracentrifugation. Cytoplasmic extracts prepared from PRL3 and control cells were fractionated across 15–45% sucrose gradients. Fractions were collected, and total RNAs were extracted from each fraction. Fig. 5A shows the absorbance of ribosomal RNAs across the gradient. The polyribosome profiles in the control and PRL3 cells indicated that PRL3-overexpressing cells had a modest decrease in polyribosomes, accompanied by accumulation of free ribosomal subunits. This pattern is indicative of a partial defect in translation initiation and would be consistent with the 20% reduction in global protein synthesis determined in the above-described pulse chase experiment. Next, mRNA levels of Csk and β-actin in polyribosomal fractions were measured by RT-PCR. Importantly, Csk mRNAs were found to shift to the monosome fractions (1–8) and lighter polyribosome fractions (9–12) in PRL3 cells (Fig. 5B). In contrast, there was minimal change in the β-actin mRNA distributions between the two cell lines. To confirm the RT-PCR results, Csk and β-actin mRNA levels along the sucrose gradient were also determined by Northern blotting. In complete agreement with the RT-PCR data, the Northern blot analysis also revealed that the Csk mRNAs shifted to the monosome and lighter polyribosome fractions in the lysates prepared from PRL3 cells (Fig. 5C). These experiments indicate that Csk is associated with fewer ribosomes in PRL3 cells, leading to lowered levels of Csk protein synthesis. This result is in full agreement with the pulse labeling experiment (Fig. 4).

**eIF2 Is Involved in the PRL3-mediated Suppression of Csk Protein Synthesis**—Overexpression of PRL3 led to a significant reduction in Csk protein synthesis, accompanied by a modest decrease in global translation. Two mechanisms that have been reported to be important for translational control involve the mTOR protein kinase and eIF2 phosphorylation. To investigate whether these pathways are integral to the mechanism by which PRL3 inhibits Csk protein synthesis, we initially considered mTOR, an important regulator of protein synthesis (32).
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To address whether the mTOR pathway is responsible for the decreased Csk translation in PRL3 cells, we treated the cells with rapamycin, an mTOR-specific small molecule inhibitor. No change in Csk protein levels were observed in control or PRL3 cells after prolonged treatment with rapamycin (data not shown), suggesting that the PRL3-mediated Csk down-regulation is unlikely to be controlled by the mTOR pathway.

We then turned our attention to elf2, which plays a key role in the initiation of protein synthesis. Phosphorylation of Ser-51 in the α-subunit of elf2 reduces the exchange of elf2-GDP for elf2-GTP that is required for recruitment of methionyl-tRNA (Met-tRNA\textsubscript{Met}) to the translation machinery. Reduction in elf2-GTP levels can significantly reduce global protein synthesis, concurrent with preferential translation of selected mRNAs encoding important stress-responsive transcription factors (33, 34). To determine the effect of PRL3 expression on elf2α phosphorylation, total cell lysates from the vector control and PRL3 cells were resolved by SDS-PAGE, electrotransferred to nitrocellulose membranes, and probed with phosphospecific elf2α antibodies. As shown in Fig. 6A, phosphorylation of the regulatory Ser-51 of elf2α in PRL3 cells was 2.4-fold higher than that in the vector control. This increase in elf2α phosphorylation would account for the partial reduction in global protein synthesis. Furthermore, these results would suggest that translation of Csk mRNA is particularly sensitive to reductions in elf2-GTP levels, a point that will be further expanded upon in the discussion.

To corroborate the finding that elf2α phosphorylation modulates Csk translation, we introduced Myc-tagged GADD34 into both the vector control and PRL3 cells. GADD34 is a regulatory subunit of protein serine/threonine phosphatase 1 (PP1) that promotes the specific dephosphorylation of elf2α by PP1 (35, 36). We reasoned that if an increase in elf2α phosphorylation is the underlying cause for reduced Csk protein synthesis, then removal of the inhibitory phosphate on Ser-51 should increase the level of Csk protein. This prediction was borne out by the outcomes of the experiments presented in Fig. 6B. Expression of GADD34 almost completely abolished pSer-51 phosphorylation on elf2α, which was accompanied by a 55% increase in the Csk protein level in PRL3 cells. Similar results were obtained when GADD34 was transiently transfected into the vector control cells (data not shown).

To ensure that the observed decrease in elf2α phosphorylation was mediated by the GADD34-PP1 holoenzyme, we also determined the status of elf2α phosphorylation when a mutant form of GADD34 lacking the C-terminal PP1-interacting domain (37) was utilized in these experiments. The mutant GADD34 is incapable of activating PP1 for elf2α dephosphorylation. Consequently, expression of the mutant GADD34 had no significant effect on elf2α phosphorylation and Csk protein levels (Fig. 6B).

We also asked whether a more physiologically stimulated elf2α phosphorylation could also inhibit Csk synthesis. To this end, HEK293 cells were treated with thapsigargin (2 μM), which is a well-characterized ER stress agent that induces endoplasmic reticulum stress (38). As expected, thapsigargin treatment significantly increased elf2α phosphorylation, which increases the translation of ATF4 mRNA (Fig. 6C), encoding a transcriptional activator of stress-responsive genes (38). Strikingly, Csk protein levels decreased significantly after 16 h of treatment with thapsigargin, indicating that Csk protein synthesis is indeed controlled by the phosphorylation status of elf2. Taken together, the data offer strong support that the elf2 kinase pathway participates in the PRL3-mediated repression of Csk protein synthesis.
Finally, to ascertain whether the PRL3 induced Csk down-regulation and the associated increase in eIF2α phosphorylation is a general phenomenon, we determined the levels of PRL3, Csk, eIF2α, and eIF2α/pSer51 in a number of permanent HEK293 cell lines overexpressing HA-tagged PRL3 (clones 11 and 14) and 3× FLAG-tagged PRL3 (clones 6 and 8). Similar to the results obtained with the PRL3 cells used in the above experiments (Fig. 6A), an increase in eIF2α phosphorylation and a decrease in Csk protein were also observed in these stable lines with elevated PRL3 expression. To establish whether the same phenomenon also occurs in cell lines that naturally overexpress PRL3, we examined three colon cancer cell lines, SW48, SW480, and SW620. SW480 and SW620 were originated from the same patient (39). Interestingly, SW48 expresses a higher migration rate than SW620 (14, 39). As shown in Fig. 7B, PRL3 is expressed to a significantly higher level in SW480 cells than in SW620 cells. Compared with SW620, SW480 cells also display a 25% reduction in Csk levels and a 2.2-fold increase in eIF2α phosphorylation. Furthermore, the level of PRL3 expression in SW48 cells is also markedly lower than that in SW480. Accordingly, the Csk protein level is 41% higher and eIF2α phosphorylation is 2-fold lower in SW480 cells. Thus, elevated expression of PRL3 in colon cancer cells also correlates with decreased Csk accumulation and increased eIF2α phosphorylation, suggesting that this mechanism may be operative in colon cancer cells as well.

**DISCUSSION**

There is increasing evidence that dysregulation of gene expression at the level of mRNA translation can contribute to cell transformation and the malignant phenotype (40). For example, activation of the Src kinase is a common theme in the development of neoplasias, and ErbB2/Her2 can boost the synthesis of Src protein through the mTOR/4E-BP1 translational pathway (41). PRL3 is a potential oncoprotein associated with tumor metastasis, and the PRL3-mediated tumorigenesis is triggered through an increase of the Src kinase activity by a mechanism involving down-regulation of Csk, a negative regulator of Src (14). In this report, we addressed the mechanism by which PRL3 can inhibit Csk. PRL3 was shown to exert a negative effect on Csk protein synthesis, rather than modulation of Csk mRNA levels or protein stability. Interestingly, the decrease in Csk protein synthesis in PRL3 cells appears to result from enhanced eIF2α phosphorylation that occurs with overexpression of PRL3. Supporting this central idea was the observation that overexpression of GADD34, a regulatory subunit for PP1 that directs dephosphorylation of eIF2α, significantly increased the synthesis of the Csk protein (Fig. 6). As described below, eIF2α phosphorylation is known to inhibit global translation. Indeed, general translation was reduced about 20% in PRL3 cells (Fig. 4B), and polyribosome analyses indicate that this diminished protein synthesis is a result of lowered translational initiation, an expected consequence of eIF2α phosphorylation. However, Csk protein synthesis was reduced 3.5-fold in PRL3 cells, as judged by pulse labeling experiments (Fig. 4). These results suggest that translation of Csk mRNA is particularly sensitive to reductions in eIF2-GTP that accompany eIF2α phosphorylation. This would suggest that there are wide variations in the translation of different mRNAs that are collectively lowered during eIF2α phosphorylation. Some mRNAs are only modestly repressed by lowered eIF2-GTP levels, while translation of others, such as Csk mRNAs, would be more dramatically inhibited. Together these studies have uncovered a previously uncharacterized role for PRL3 and eIF2α phosphorylation in the gene-specific translational control of Csk expression.

Translational control in eukaryotes is primarily exerted at the level of initiation through phosphorylation of eIF2α (33, 34, 42). A family of eIF2α kinases has been identified that are each activated by unique stress conditions. The eIF2α kinases include PEK/Perk, which is triggered by accumulation of misfolded protein in the ER, so-called ER stress, GCN2, which is activated by amino acid starvation or UV irradiation, HRI that is induced by heme deprivation or oxidative stress, and PKR that participates in an anti-viral defense pathway. Phosphorylation of eIF2α reduces general translation by lowering the levels of eIF2-GTP that are required for ribosomal acquisition of Met-tRNA<sub>Met</sub>. Lowered global translation would conserve cellular resources during various stress conditions. In addition to a general role in translational initiation, there have been reports that eIF2 could also mediate gene-specific regulation of protein synthesis (42). For example, coincident with lowered proteins synthesis, eIF2α phosphorylation enhances the preferential translation of ATF4 mRNA, encoding a basic zipper (bZIP) transcription activator for stress responsive genes (36, 38).

Measurements of total protein synthesis indicate that translation of the vast majority of mRNAs is repressed in response to eIF2α phosphorylation. However, this study suggests that translation of these different mRNAs can have a range of sensitivities to lowered eIF2-GTP. Whereas PRL3 cells displayed enhanced eIF2α phosphorylation that led to only a 20% reduction in global protein synthesis, translation of Csk mRNA was...
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translational control machinery may be important for the development of new anticancer therapies.

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