Estrogen receptor α promotes protein synthesis by fine-tuning the expression of the eukaryotic translation initiation factor 3 subunit f (eIF3f)

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Approximately two thirds of all breast cancer cases are estrogen receptor (ER)–positive. The treatment of this breast cancer subtype with endocrine therapies is effective in the adjuvant and recurrent settings. However, their effectiveness is compromised by the emergence of intrinsic or acquired resistance. Thus, identification of new molecular targets can significantly contribute to the development of novel therapeutic strategies. In recent years, many studies have implicated aberrant levels of translation initiation factors in cancer etiology and provided evidence that identifies these factors as promising therapeutic targets. Accordingly, we observed reduced levels of the eIF3 subunit eIF3f in ER-positive breast cancer cells compared with ER-negative cells, and determined that low eIF3f levels are required for proper proliferation and survival of ER-positive MCF7 cells. The expression of eIF3f is tightly controlled by ERα at the transcriptional (genomic pathway) and translational (nongenomic pathway) level. Specifically, estrogen-bound ERα represses expression of the EIF3F gene, while promoting eIF3f mRNA translation. To regulate translation, estrogen activates the mTORC1 pathway, which enhances the binding of eIF3 to the eIF4F complex and, consequently, the assembly of the 48S preinitiation complexes and protein synthesis. We observed preferential translation of mRNAs with highly structured 5′-UTRs that usually encode factors involved in cell proliferation and survival (e.g. cyclin D1 and survivin). Our results underscore the importance of estrogen-ERα–mediated control of eIF3f expression for the proliferation and survival of ER-positive breast cancer cells. These findings may provide rationale for the development of new therapies to treat ER-positive breast cancer.

Breast cancer is the leading cause of cancer-related deaths among females worldwide. Approximately 70% of breast cancers are estrogen receptor (ER)² positive, which underscores the dependence of cancer cells on estrogen for growth and survival (1). ER-positive breast cancers are usually treated with endocrine therapies that inhibit ER function either by antagonizing the binding of estrogen to ER (selective estrogen receptor modulators, e.g. tamoxifen), promoting ER degradation (selective estrogen receptor degraders, e.g. fulvestrant), or blocking estrogen biosynthesis (aromatase inhibitors, e.g. letrozole, anastrozole, and exemestane) (2). However, their effectiveness is compromised by the emergence of intrinsic or acquired resistance in treated patients (2–4). Therefore, better understanding of ER-positive breast cancer biology is critical to development of more effective therapeutic strategies that minimize resistance and cancer recurrence.

ERα is a nuclear receptor whose activity is primarily regulated by the binding of its ligand estrogen (17β-estradiol). Estrogen-ERα complex acts as a transcription factor that activates or represses the expression of multiple target genes (genomic pathway) (5, 6). Alternatively, extranuclear ligand-bound ER elicits rapid, stimulatory effects on cytoplasmic signaling pathways mediated by the mitogen-activated protein kinase (MAPK)/ERK or the phosphatidylinositol 3-kinase (PI3K)/AKT/mTOR complex 1 (mTORC1), also termed the nongenomic pathway (7). By acting through these signaling pathways, increased levels of estrogen-ERα complex promote cell proliferation, cell cycle progression, survival, angiogenesis, invasion, and migration in cancer cells.

Regulation of mRNA translation is critical to define the proteome, maintain homeostasis, and control cell proliferation, growth, and development. Protein synthesis occurs in four steps: initiation, elongation, termination, and ribosome recycling, with initiation being the rate-limiting phase (8). The translation initiation comprises: (a) the assembly of the eukaryotic translation initiation factor 4F complex (eIF4F) composed of the cap-binding protein eIF4E, the RNA helicase eIF4A, and the scaffolding protein eIF4G on the 5′ cap-structure of cellular mRNAs; (b) the formation of the 43S preinitiation complex (PIC) that consists of the 40S ribosomal subunit, translation initiation factors eIF1, eIF1A, eIF3, eIF5, and the ternary complex that includes eIF2, initiator Met-tRNA⁰⁰, and GTP; (c) the preinitiation complex; (d) the 43S preinitiation complex; and (e) the 60S subunit.

This work was supported by American Cancer Society Grant RSG-13-287-01-TBE; NIGMS, National Institutes of Health Grant GM128675; NCI, National Institutes of Health Grant CA151112; and a research award from the Atol Charitable Trust (to M. K. H.). The authors declare that they have no conflicts of interest with the contents of this article. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

This article contains supporting "Experimental procedures," Figs. S1 and S2, and Table S1.

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²The abbreviations used are: ER, estrogen receptor; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; PIC, preinitiation complex; YLC, yeast-like core; ERE, estrogen response element; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; mTOR, mechanistic target of rapamycin; RT-qPCR, quantitative reverse transcription PCR.
the recruitment of the 43S PIC to the mRNA via the interaction of eIF3 with eIF4G to form the 48S PIC; (d) the scanning of the 5′-UTR; and (e) the assembly of the 80S ribosome-initiation complex at the AUG start codon (9). Translation initiation is tightly regulated by the oncogenic MAPK/ERK and PI3K/AKT/mTORC1 signaling pathways (10). The mechanisms by which mTORC1 controls this phase have been well-studied. They involve (a) phosphorylation of translational inhibitor 4E-binding protein 1 (4E-BP1), which prevents its interaction with eIF4E and facilitates eIF4E binding to eIF4G to form the eIF4F cap-binding complex, and (b) eIF3-mediated activation of mTOR effector, the 40S ribosomal kinase 1 (S6K1), which results in enhanced assembly of the 48S PIC and, therefore, increased protein synthesis (10, 11).

Dysregulation of mRNA translation is observed in cancer. Altered levels of translation initiation factors and/or the activation of the upstream oncogenic pathways increase global protein synthesis and/or the translation of specific mRNAs. These translational changes promote malignant transformation and tumor development (10, 12–15). Many studies have demonstrated the contribution of altered eIF4F levels and activity to cancer etiology. Targeting eIF4F components or the upstream signaling pathways (e.g. PI3K and mTOR inhibitors) has shown promising results in preclinical studies and clinical trials (16–19). However, ineffectiveness or resistance in monotherapy setting and/or toxicity in combination limited their clinical utility.

Here we focus on expanding our understanding of the role of the translation initiation factor eIF3 in tumor biology. eIF3 is a large complex composed of 13 nonidentical subunits (eIF3a-m) in human cells. Current model proposes that the assembly of eIF3 starts with the interaction of eIF3a and eIF3b to form the eIF3 nucleation core. The association of eIF3g and eIF3i to eIF3b gives rise to the subcomplex known as yeast-like core (YLC). Then, the sequential interaction of the seven subunits eIF3c, eIF3e, eIF3f, eIF3g, eIF3i, and eIF3m with eIF3a forms the eIF3 octamer. The nonoctameric eIF3d subunit joins eIF3 complex through its binding to eIF3e (20). Overexpression of eIF3a, eIF3b, eIF3c, eIF3f, eIF3i, and eIF3m, or underexpression of eIF3e and eIF3f have been reported in several cancers, including breast tumors (12, 15, 21). First evidence supporting a role for eIF3 in cancer biology was obtained by ectopic overexpression of individual subunits in NIH3T3 cells. Ectopic expression of eIF3a, eIF3b, eIF3c, eIF3h, and eIF3i stimulates global protein synthesis and translation of mRNAs that encode growth-regulating proteins, and leads to malignant transformation. In contrast, ectopic expression of eIF3e and eIF3f inhibits protein synthesis and decreases cell growth and proliferation (22). Recent studies have demonstrated that changes in the levels of a single eIF3 subunit can affect the expression of other subunits and result in the formation of eIF3 subcomplexes responsible for the translation of specific set of mRNAs (23). Additionally, Cate and colleagues (24, 25) have shown that eIF3 binds to a specific group of mRNAs involved in cell growth control and promotes their cap-dependent translation independently of eIF4E activity. These results indicate that altered levels of individual eIF3 subunits and, therefore, the formation of the eIF3 complex and/or subcomplexes significantly define the translational landscape of cancer cells.

To investigate the role of eIF3 in breast cancer biology, we have determined the levels of eIF3a, eIF3b, and eIF3f in a panel of breast cancer cells. Interestingly, we observed lower levels of eIF3f in ER-positive cells compared with ER-negative cells. Our studies demonstrate that genomic and nongenomic estrogen-ERα pathways coordinately fine-tune the expression of eIF3f in ER-positive MCF7 cells. This tight regulation buffers the levels of eIF3f to ensure the synthesis of factors required for estrogen-dependent cell proliferation and survival.

Results

Levels of eIF3f are significantly reduced in ER-positive compared with ER-negative breast cancer cells

Altered amounts of individual eIF3 subunits have been detected in breast tumors (15, 21, 26, 27). Particularly, elevated levels of eIF3a and eIF3b and reduced levels of eIF3f are observed in breast cancers (21). eIF3a and eIF3b subunits serve as the nucleation core around which other subunits assemble to form the eIF3 complex, and eIF3f plays a critical role in stabilizing the complex (23). To investigate the role of these proteins in breast cancer biology, we first evaluated their expression in a panel of ER-positive (MCF7, T47D, ZR75.1, and MDA-MB-361) and ER-negative (BT-474, MDA-MB-231, and MDA-MB-436) breast cancer cells (Fig. 1). Interestingly, we observed a notably reduced expression of eIF3f in ER-positive cells compared with ER-negative cells. However, the expression levels of eIF3a were quite similar among cell lines, and eIF3b levels were significantly higher in BT-474 cells compared with the ER-positive and MDA-MB-231 cell lines (p < 0.05) (Fig. 1B). Our results suggested that eIF3f expression in ER-positive breast cancer cells may be a function of the estrogen-ER pathway.

Estrogen represses the transcription of the eIF3F gene

We first asked whether EIF3F gene is a direct target of the genomic estrogen-ER pathway. We measured the levels of the eIF3f mRNA in response to vehicle or estrogen stimulation in MCF7 cells by RT-qPCR (Fig. 2A, right plot). We found a significant reduction in the amount of the eIF3f mRNA starting at 12 h of estrogen treatment. As expected, the levels of the TFF1 mRNA, a well-characterized ER target gene, significantly increased at earlier time points (6 h) (Fig. 2A, left plot). In contrast, the amount of the eIF3a mRNA did not change and the levels of the eIF3b mRNA only showed a significant increase at the 6-h time point (Fig. S1).

To rule out the possibility that estrogen affects the stability of the eIF3f mRNA, we determined its half-life. In vehicle- and estrogen-stimulated MCF7 cells, the half-life of the eIF3f mRNA was ~12 h (Fig. 2B, right plot). Interestingly, we found that estrogen treatment significantly stabilized the TFF1 mRNA, in addition to positively regulating TFF1 transcription (Fig. 2B, left plot). Next, we investigated the effect of tamoxifen on EIF3F transcription. As expected, tamoxifen antagonized estrogen-mediated activation of TFF1 transcription, but, like estrogen, it repressed EIF3F transcription (Fig. 2C). Additionally, we confirmed that neither estrogen nor tamoxifen affects the amount of the eIF3f mRNA in ER-negative MDA-MB-231 cells (Fig. 2C). Thus, estrogen induces transcriptional repression of the EIF3F gene.
ERα regulates expression of eIF3f

To confirm that ERα directly affects the observed response to estrogen, we performed specific silencing of ERα and determined the levels of the eIF3f mRNA in control or ERα-silenced MCF7 cells treated with vehicle or estrogen for 24 h. As shown in Fig. 3A, suppression of ERα expression prevented ligand-induced repression of EIF3F transcription. As expected, we detected reduced levels of the TFF1 mRNA in estrogen-stimulated ERα-silenced cells compared with control cells. In addition, we confirmed ERα silencing by immunoblotting (Fig. 3B). Interestingly, the amount of the eIF3f protein did not significantly change under any experimental condition, although mRNA levels did (Fig. 3 and Fig. S2). These results suggest that the decrease in eIF3f mRNA levels by the estrogen-ERα complex is buffered by an increase in estrogen-stimulated mRNA translation, as demonstrated below. In contrast, we did not observe any effect of estrogen on the translation of the eIF3a and eIF3b mRNAs (Fig. 3B and Figs. S1 and S2).

The mechanisms by which estrogen-bound ERα represses transcription are poorly understood. It has been proposed that early gene inhibition involves transient binding of ERα to the promoter and sequestration of limiting factors away from the repressed gene, a mechanism known as physiologic squelching. However, late gene inhibition (after 6 h of treatment) requires the binding of ERα and repressors or corepressors at sites adjacent to the repressed gene. Some reports indicate that the expression of specific repressors is induced by estrogen, which explains the lag (5, 28). The results shown in Fig. 2A suggested that estrogen-mediated repression of EIF3F transcription might occur according to the late inhibition model. However, we did not observe any change in the amount of eIF3f transcripts when estrogen-stimulated cells were pretreated with the protein synthesis inhibitor cycloheximide (Fig. 4A). These results indicated that estrogen-induced expression of a repressor was not required for EIF3F repression, and suggested regulation by physiologic squelching. To test this hypothesis, we performed chromatin immunoprecipitation (ChIP) assays using nuclear extracts from MCF7 cells treated with vehicle or estrogen for 30 min (Fig. 4B). For these studies, we used three pairs of primers to amplify different regions of the EIF3F promoter, and another pair that amplified an upstream region containing an experimentally identified ER binding site (ERE). Distal ERs are involved in late gene inhibition (5). As expected, we did not detect increased binding of ERα to this distal ERE upon estrogen stimulation. In contrast, we observed elevated binding of ERα to the EIF3F promoter in response to estrogen treatment, particularly to the region proximal to the transcription start site, which correlated with reduced loading of RNA polymerase II (Fig. 4B). However, these changes were much less pronounced compared with the differential binding observed at the TFF1 promoter, which contains a canonical ERE (Fig. 4C). These results indicated a transient and labile interaction of ERα with the EIF3F promoter and supported a physiologic squelching mechanism for estrogen-ERα repression of EIF3F transcription. Although transcription inhibition may occur as early as 30 min upon estrogen stimulation, changes in eIF3f mRNA levels were detectable at later times because of its ∼12-h half-life (Fig. 2B).

Estrogen-ERα pathway promotes the binding of eIF3 to eIF4F and increases cap-dependent translation

We observed that estrogen-induced reduction of the eIF3f mRNA levels was not associated with a decrease in the eIF3f protein amount (Figs. 2A and 3, A and B). This result suggested that eIF3f expression is buffered by up-regulation of eIF3f mRNA translation in response to estrogen. This effect may be mediated by the mTORC1 pathway, which is activated in estrogen-treated MCF7 cells as indicated by the increased phosphorylation of its downstream targets S6K1 and 4E-BP1 (Fig. 3B). mTORC1 is activated by the nongenomic estrogen-ER pathway and, as previously described, is a key regulator of cap-dependent translation (7, 10). To test this hypothesis, we evaluated protein synthesis using a Dual-Luciferase reporter system that monitors the ratio between cap-dependent and cap-independent translation initiation (Fig. 5A). We observed that estrogen
stimulation increased cap-dependent translation by ~ 2-fold, which was prevented by pretreatment of the cells with either mTORC1 (rapamycin) or ERα (fulvestrant) inhibitors (Fig. 5A). Additionally, we found that, unlike estrogen, tamoxifen failed to activate mRNA translation (Fig. 5A). These results support a role of estrogen-ERα signaling in the control of mRNA translation through the activation of mTORC1.

mTORC1 regulates the formation of the cap-binding complex eIF4F and its interaction with eIF3 to form the 48S preinitiation complex (10, 11, 29). Accordingly, we postulated that estrogen may enhance translation by promoting the assembly of the 48S PIC. To test this hypothesis, we isolated translation initiation complexes using m7GTP-agarose beads from MCF7 cells grown in DMEM containing 5% charcoal-stripped FBS and supplemented with vehicle or estrogen. As shown in Fig. 5B, the binding of eIF4G and the eIF3 subunits eIF3a, eIF3b, and eIF3f to eIF4E increased in response to estrogen stimulation, whereas 4E-BP1 interaction with eIF4E decreased. As expected, inhibition of mTORC1 with rapamycin promoted the binding of dephosphorylated 4E-BP1 to eIF4E and, consequently, the dissociation of eIF4G and the eIF3 subunits from eIF4E. Additionally, we observed that inhibition of ER activity with fulvestrant or tamoxifen prevented estrogen-induced interaction of the eIF3 subunits with the cap-binding complex eIF4F (Fig. 5B).
and Table S1). However, we found no effect of estrogen on the assembly of the elf3a, elf3b, and elf3f subunits (Fig. 5C). These results indicated that estrogen-mediated activation of mTORC1 facilitates the binding of elf3f and, therefore, the 43S PIC to the cap-binding complex elf4F. This increase in the levels of the preinitiation complexes specifically promotes translation of mRNAs harboring long and structured 5′-UTRs that encode factors required for cell proliferation, survival, or angiogenesis (12). Accordingly, we observed enhanced synthesis of cyclin D1 and survivin in estrogen-stimulated cells, which is reversed by the treatment with the inhibitors of mTORC1 or estrogen receptor (Fig. 5D). However, the amount of the anti-apoptotic factor Mcl1, whose translation required high levels of phosphorylated elf4E, did not change under any condition (Fig. 5D) (30). As predicted, the levels of elf3f protein were similar in vehicle- and estrogen-stimulated cells, but they decreased after 36 h of treatment with rapamycin or tamoxifen (Fig. 5E).

Strikingly, we detected the interaction between Erα and the elf3 complex in unstimulated MCF7 cells, which was dissociated by the treatment with estrogen. Using immunoprecipitation assays, we observed an increased binding of Erα to elf3b and elf3f in the absence of estrogen (3.5- and 1.6-fold over background, respectively), which was prevented by estrogen stimulation (Fig. 6, A–C). Ligand binding to Erα induces a conformational change that promotes Erα dimerization and activation and determines its association with transcriptional coactivators and its dissociation from inhibitory chaperones (31, 32). Consistently, this structural change may also prevent the binding of Erα to elf3. In addition, estrogen-bound Erα translocates to the nucleus, where it activates or represses transcription of target genes. Accordingly, we observed estrogen-induced accumulation of Erα in nuclear extracts, but cellular distribution of elf3b and elf3f did not change (Fig. 6D). These results suggested that estrogen-ligated Erα may also facilitate the interaction of elf3f with other translation initiation factors.

**elf3f overexpression prevents estrogen-induced up-regulation of protein synthesis, reduces cell proliferation, and induces apoptosis**

Down-regulation of elf3f is also observed in melanoma and pancreatic tumors. In cell derived from these tumors, ectopic expression of elf3f results in the inhibition of protein synthesis, reduced cell proliferation, and increased apoptosis (27). Similar effects are detected in immortalized NIH3T3 fibroblasts in response to elf3f overexpression (22). These results underscore the importance of a tight control of elf3f levels for proper cellular function. To confirm the role of estrogen-Erα–mediated regulation of elf3f in ER-positive MCF7 cell biology, we evaluated the rate of protein synthesis, proliferation, and apoptosis in cells expressing the HA tag or HA-tagged elf3f. mRNA translation was tested using the Dual-Luciferase reporter system, as described in Fig. 5A. As expected, estrogen stimulation enhanced cap-dependent translation in HA-expressing cells, but it did not have any effect on translation in cells expressing HA-elf3f (Fig. 7A). Consistently, ectopic expression of elf3f also prevented estrogen-induced synthesis of cyclin D1 and survivin (Fig. 7B). Estrogen stimulates the transcription of the CCND1 gene, which could explain the lower effect of elf3f overexpression on cyclin D1 expression compared with survivin (33). These results corroborated the inhibitory effect of high elf3f levels on protein synthesis in MCF7 cells. Then, we investigated the mechanism by which elf3f overexpression affects protein synthesis. Because estrogen promoted the binding of elf3 to elf4f, we first examined the interaction of elf3b with elf3a and elf3f, and their association with the cap-binding complex elf4F in HA- or HA-elf3f–expressing cells. As shown in Fig. 7, C and D, ectopic expression of elf3f did not interfere with the assembly of the translation initiation complex. We did not observe any effect on mTORC1 activity either, because phosphorylation of the ribosomal protein S6 and 4E-BP1 was not affected. Alternatively, elf3f overexpression might alter the expression of other elf3 subunits, the assembly of elf3 complexes, the formation of the 43S PIC, or other translation-related functions of elf3f as discussed below. Further studies need to be performed to evaluate these options.

Next, we determined the effect of ectopic expression of elf3f on cell proliferation using MTT cell viability assays. MCF7 cells transiently expressing HA tag (control) or HA-elf3f (elf3f) were grown in phenol red–free DMEM supplemented with 5% charcoal-stripped FBS and estrogen (100 nM) for 0, 2, and 4 days. As shown in Fig. 7E, we observed proliferation of control cells; however, cell viability decreased in elf3f-overexpressing...
cells, particularly in the first 2 days. This effect correlated with a marked reduction in the levels of the HA-eIF3f protein (Fig. 7E). These results indicated that the overexpression of eIF3f induced apoptosis. Accordingly, we observed a significantly higher fraction of early and late apoptotic cells in eIF3f-expressing cells than in HA-expressing cells after double staining with Annexin V-PE and 7-AAD (Fig. 7F). As control, cells transfected with the empty vector were treated with the vehicle or etoposide to induce apoptosis. Surprisingly, we observed that eIF3f overexpression was more efficient in inducing apoptosis than etoposide in MCF7 cells.

All together, these results indicate that the levels of eIF3f are critical to ensure proper synthesis of proteins required for cell proliferation and survival, such as cyclin D1 and survivin in estrogen-stimulated MCF7 cells. Therefore, we conclude that a tight and coordinated control of eIF3f expression by estrogen-ERα genomic and nongenomic pathways is essential for adequate proliferation and survival of ER-positive cells.

Discussion

ERα-positive breast tumors rely on estrogen-ERα activity for their development and progression. Through its genomic and
Figure 5. Estrogen-ERα pathway facilitates the binding of eIF3 to eIF4F and promotes cap-dependent translation. A, MCF7 cells transfected with R-HCV-L bicistronic plasmid were grown in phenol red–free DMEM containing 5% charcoal-treated FBS for 3 days before being treated with vehicle, estradiol (100 nM), estradiol and rapamycin (20 nM), estradiol and fulvestrant (100 nM), or tamoxifen (100 nM) for 24 h. Cell extracts were obtained, and Renilla and Firefly Luciferase activities were determined. Renilla/Firefly ratios were calculated and mean ± S.E. of three independent experiments were plotted relative to vehicle-treated cells (set to 1) (*, p ≤ 0.05). B, MCF7 cells were estrogen-deprived for 3 days. Cells were treated with vehicle, rapamycin (20 nM), or fulvestrant (100 nM) for 30 min before being stimulated with estradiol (100 nM) or tamoxifen (100 nM) for 2 h. Cell extracts were obtained and translation initiation complexes isolated using m7GTP-agarose beads. Indicated proteins were analyzed by immunoblotting. C, cell extracts obtained as in B were incubated with anti-eIF3b antibody overnight at 4 °C. Purified immunocomplexes were resolved by SDS-PAGE, and indicated proteins were analyzed by immunoblotting. D, MCF7 cells were cultured and treated for 24 h as in B. Cell extracts were prepared and resolved by SDS-PAGE, and indicated proteins were detected by immunoblotting. E, MCF7 cells were grown in phenol red–free media supplemented with 5% charcoal-stripped FBS for 3 days before stimulation with vehicle (EtOH), estradiol (100 nM), estradiol and mTOR inhibitor pp242 (2.5 μM), or tamoxifen (100 nM) for 24 or 36 h. Cell lysates were prepared and indicated proteins were analyzed by immunoblotting.

Figure 6. Estrogen induces the dissociation of ERα from eIF3 and the nuclear localization of ERα. A, HA-transfected or HA-eIF3f-transfected MCF7 cells were incubated in low-estrogen DMEM for 3 days, followed by stimulation with estrogen (10 nM) for 30 min. Cell extracts were prepared, precleared with protein G–agarose beads for 1 h at 4 °C, and incubated with anti-HA antibody overnight at 4 °C. Isolated immunocomplexes were resolved by SDS-PAGE and indicated proteins were detected by immunoblotting. B and C, MCF7 cells were treated as in A, and cell extracts were precleared with protein A/G–agarose for 1 h at 4 °C followed by incubation with anti-ERα (B) or anti-eIF3b (C) antibodies overnight at 4 °C. Isolated immunocomplexes were resolved by SDS-PAGE and indicated proteins were analyzed by immunoblotting. D, MCF7 cells were estrogen-deprived for 3 days, followed by stimulation with estrogen (10 nM) for 1 h. Nuclear and cytoplasmic extracts were generated as described in “Experimental procedures” and separated by SDS-PAGE. Indicated proteins were analyzed by immunoblotting.
nongenomic pathways, ER enhances the transcription and the activity of multiple factors that promote cell proliferation, cell cycle progression, angiogenesis, and survival (7). In this report, we demonstrated that estrogen-bound ERα/H9251 also regulates mRNA translation and, therefore, controls the expression of target genes such as *CCND1* at translational level. This regulation occurs by two mechanisms in MCF7 cells: First, by maintaining proper levels of the eIF3f subunit (Figs. 2 and 3) and second, by activating the mTORC1 pathway to facilitate the assembly of the 48S preinitiation complex through the interaction of eIF3 and eIF4G (Fig. 5). Additionally, we found that elevated levels of eIF3f reduce the rate of protein synthesis, decrease proliferation, and increase apoptosis (Fig. 7). These results suggest that eIF3f may be a negative regulator of cancer cell growth. Therefore, any therapeutic strategy aiming to induce eIF3f expression may be effective for treatment of ER-positive breast cancers, particularly those with low eIF3f levels.

We detected reduced levels of the eIF3 subunit eIF3f in ER-positive breast cancer cells compared with ER-negative cells (Fig. 1). In agreement with this observation, overexpression of
Fig. 7, mRNAs (eIF3 subunits leads to the formation of partial eIF3 subcomplexes, by which these changes contribute to cancer etiology. However, the mechanisms by which these changes contribute to cancer etiology are still being elucidated.

Some evidence indicates that imbalanced expression of the eIF3 subunits leads to the formation of partial eIF3 subcomplexes, which may control translation of a specific set of mRNAs (20, 21). Thus, ectopic expression or siRNA knockdown of eIF3a raises or reduces the protein levels of most of the other eIF3 subunits, respectively, which in turn affect the formation and stability of the entire eIF3 complex (22, 34). These changes in eIF3a levels also alter the translation of specific mRNAs. Consequently, the synthesis of tyrosinated α-tubulin, the ribonucleotide reductase regulator M2, and the N-myc downstream regulated gene-1 (NDRG1) is stimulated in cell overexpressing eIF3a, whereas levels of p27kip1 decrease. Similar effects are observed on other mRNAs that may be responsible for the regulation of cisplatin sensitivity and DNA repair activities. Low levels of eIF3a reverse these changes (21). Another example is eIF3h, whose knockdown induces down-regulation of the eIF3k and eIF3l subunits. High levels of eIF3h are observed in prostate and breast cancers, and eIF3h overexpression in NIH3T3 cells enhances the synthesis of cyclin D1, ODC, and FGFR2 (23, 35). The imbalanced expression of eIF3 subunits and the formation of eIF3 subcomplexes may alter the canonical function of eIF3 in the assembly of the 48S PIC through its interaction with eIF4G. Other translational steps in which eIF3 plays a critical role (e.g., translation initiation, termination, and ribosome recycling) may be also affected. Remarkably, Cate and colleagues identified a group of mRNAs whose 5′-UTRs specifically cross-linked to eIF3a, eIF3b, eIF3d, and eIF3g. Using c-JUN mRNA, they found that the binding of eIF3 subunits to specific secondary structures at its 5′-UTRs promotes the interaction of eIF3d to the 5′ cap and eIF4F-independent recruitment of the mRNA to the 43S PICs for translation initiation (24, 25). These results indicate that the levels of the entire eIF3 complex and/or partial eIF3 subcomplexes may be critical to ensure the proper synthesis of factors required for the proliferation, growth, and survival of cancer cells.

Silencing of eIF3f significantly reduces the levels of eIF3h, eIF3m, eIF3k, and eIF3l subunits, which impairs the formation of the eIF3 octamer and the entire complex. However, the assembly of YLC complex (eIF3a, eIF3b, eIF3g and eIF3l) is not affected. This complex represents a minimal functional unit of eIF3 able to promote the binding of ternary complex and mRNA to the 40S ribosome and the scanning of the 5′-UTR of mRNAs (23, 34). These results suggest that the levels of entire eIF3 complex may be reduced, whereas YLC-like subcomplexes may be accumulated in cells containing low amounts of eIF3f such as MCF7 cells. We observed the association of eIF3f to eIF3a and eIF3b and their binding to eIF4F, which is mediated by eIF3c, eIF3e and eIF3d (Fig. 5, B and C) (36). Our results confirm the presence of the entire functional eIF3 complex in MCF7 cells, whose estrogen-stimulated binding to eIF4F enhances cap-dependent mRNA translation, including the synthesis of cell cycle and survival regulators such as cyclin D1 and survivin (Fig. 5, A and D). Further studies will be performed to investigate the existence of eIF3 subcomplexes, as well as their implication in the translation of a specific group of mRNAs. Altered eIF3f levels affect the synthesis of proteins involved in cell proliferation and survival such as cyclin D1 and survivin, and result in reduced proliferation and increased apoptosis in MCF7 cells (Fig. 7, B, E, and F). Consequently, we expect to identify other tumor-promoting factors, whose expression is sensitive to eIF3f levels. Similar effects are observed in other cancer cells containing low levels of eIF3f such as melanoma and pancreatic cancer cells (27, 37). The mechanisms by which increased levels of eIF3f inhibit protein synthesis are not well-known. Some studies indicate that ectopic expression of eIF3f promotes degradation of the 28S and 18S rRNAs, resulting in reduced number of ribosomes and, therefore, decreased rate of protein synthesis (27, 37). Consequently, cells undergo apoptosis, which in turn induces the activation of CDK11 p46, a kinase that phosphorylates eIF3f and stabilizes its binding to other eIF3 subunits into an inactive complex (38). Our studies did not show changes in the levels of 18S rRNA by RT-qPCR (data not shown), altered binding of eIF3f to eIF3a and eIF3b, or impaired association of eIF3f with eIF4F in MCF7 cells overexpressing eIF3f, compared with HA-expressing cells (Fig. 7, C and D); however, we cannot rule out this mechanism. In fact, estrogen-ER pathway has been implicated in the regulation of ribosome biosynthesis (39).

In addition to its function as a stabilizer of the eIF3 complex, eIF3f plays a critical role in the regulation of translation initiation by the mTORC1 pathway in certain cell lines. Thus, eIF3f works as a scaffolding protein for the activation of S6K by mTORC1, which results in the phosphorylation of components of the translational machinery and, consequently, increased assembly of the 48S PIC, as described above (11, 40). Therefore, eIF3f levels may modulate mTORC1 activity in translation initiation. However, we did not observe changes in mTORC1 activity or 48S PIC assembly in MCF7 cells overexpressing eIF3f (Fig. 7, B–D). Interestingly, cell lines with low eIF3f levels (MCF7, T47D, and ZR75.1) harbor activating alterations in components of the PI3K/AKT/mTORC1 pathway, and increased eIF3f levels may not have a significant impact on downstream targets. In contrast, high levels of eIF3f in cells
ERα regulates expression of eIF3f

without up-regulated mTORC1 pathway (MDA-MB-231, BT-474, and MDA-MB-436) may significantly contribute to translation initiation (Fig. 1) (41).

As discussed, low levels of eIF3f are essential to keep the proper expression of proteins involved in cell proliferation and survival in melanoma, pancreatic, and breast cancer cells. Although allelic loss of the EIF3F gene accounts for reduced expression in melanoma and pancreatic cancers, gene amplification or deletion or mutations of this gene are not frequently detected (< 0.9%) in the breast tumor samples available in The Cancer Genome Atlas (TCGA). These data highlight the relevant role of the estrogen-ERα genomic and nongenomic pathways in the regulation of eIF3f expression in MCF7 cells. By describing this regulatory mechanism, we contribute to a better understanding of ER-positive MCF7 breast cancer cell biology and provide rationale for the investigation of eIF3f and/or eIF3f as druggable targets. Our results may also provide insight into the response of ER-positive cells to endocrine therapies. In agreement with this notion, we observed that tamoxifen reduces eIF3f mRNA and protein levels, which was associated with protection from drug-induced apoptosis in melanoma cells (Figs. 2C and 5E) (27). These results suggest that tamoxifen may not be as effective as other endocrine therapies for the treatment of ER-positive tumors with low eIF3f levels.

Experimental procedures

Cell culture and reagents

All cell lines were originally obtained from the ATCC. MCF7, MDA-MB-231, BT-474, T47D, MDA-MB-361, and ZR75.1 were maintained in DMEM (Corning), supplemented with 10% FBS (Atlanta Biologicals), and penicillin/streptomycin (Corning). MDA-MB-436 cells were maintained in DMEM:Ham’s (1:1) (Corning) supplemented with 10% FBS, l-glutamine (2 mM) (HyClone), and penicillin/streptomycin. For estrogen stimulation experiments, cells were grown in phenol red–free DMEM (Corning) with 5 or 10% charcoal-stripped FBS (Atlanta Biologicals) for 3 days. β-Estradiol (Sigma-Aldrich), rapamycin (Sigma-Aldrich), pp242 (EMD Millipore), (Z)-4-hydroxynortamoxifen (Sigma-Aldrich), fulvestrant (Sigma-Aldrich), actinomycin D (Sigma-Aldrich), etoposide (Sigma-Aldrich), droxytamoxifen (Sigma-Aldrich), fulvestrant (Sigma-Aldrich), rapamycin (Sigma-Aldrich), pp242 (EMD Millipore), (Z)-4-hydroxytamoxifen (Sigma-Aldrich), fulvestrant (Sigma-Aldrich), actinomycin D (Sigma-Aldrich), etoposide (Sigma-Aldrich), and cycloheximide (Sigma-Aldrich) were used as indicated in figure legends.

Plasmids and transfections

Control siRNA and siRNAs against ERα were described previously (42). pKH3-HA-eIF3f plasmid was generated by inserting eIF3f cDNA in-frame into pKH3-HA vector. p-RL-HCV-FL reporter plasmid and pcDNA3-HA plasmid were described (43, 44).

MCF7 cells were transfected using FuGENE HD (Promega) according to the manufacturer’s protocol. Lipofectamine RNAiMAX (Invitrogen) was used for transfection of siRNAs following the manufacturer’s protocol.

Immunoblot analysis, immunoprecipitation, and cap-binding assays

Cell lysates were prepared by incubating the cells in ice-cold lysis buffer (10 mM K3PO4, 1 mM EDTA, 10 mM MgCl2, 5 mM EGTA, 50 mM β-glycerophosphate, 50 mM NaF, 0.5% Nonidet P-40, 0.1% Brij, 40 μg/ml PMSF, 10 μg/ml leupeptin, 5 μg/ml pepstatin, and 10 μg/ml aprotinin) for 20 min on ice, followed by centrifugation at 10,000 rpm at 4 °C for 10 min. Equal amounts of whole-cell extracts were resolved by SDS-PAGE (4–12% gradient) and transferred to nitrocellulose membrane. Indicated proteins were detected by immunoblot analysis using specific antibodies: Anti-phospho-S6K (Thr-389), anti-phospho-S6 (Ser-235/236), anti-phospho-4EBP1, anti-phospho-4E-BP1 (Ser-65), anti-eIF4G, anti-eIF4E, anti-eIF4A, anti-lamin A/C, anti-Mcl1, anti-cyclin D1, anti-survivin, and anti-TFF1 (Cell Signaling Technology); anti-actin (C-11), anti-eIF3a (eIF3α H-300), anti-eIF3b (eIF3B H-328), anti-eIF3f (eIF3f H-4), and ERα (HC-20) (Santa Cruz Biotechnology); anti-HA (clone HA-7; Sigma-Aldrich). Anti-goat IRDye, anti-mouse IRDye, and anti-rabbit IRDye (LI-COR Biosciences) were used as secondary antibodies.

For immunoprecipitation assays, equal amounts of protein were pre-cleared with 20 μl of 50% protein A– or protein G–agarose bead slurries for 1 h at 4 °C followed by incubation with anti-eIF3b (10 μl), anti-ERα (10 μl), or anti-HA (2 μl) overnight at 4 °C. Protein A– or protein G–agarose beads, previously blocked with BSA, were added to the samples and incubation continued for 1 h at 4 °C. Beads were washed five times with lysis buffer for 5 min at 4 °C and collected by centrifugation at 3,000 rpm for 3 min. Immunocomplexes were resolved and detected as described above.

For cap-binding assays, equal amounts of protein were incubated with m7GTP-agarose (Creative BioMart; m7GTP-001A) overnight at 4 °C. Beads were washed five times with lysis buffer, and complexes were resolved and detected as described above.

Nuclear-cytoplasmic fractionation

Nuclear and cytoplasmic extracts were prepared using NE-PER™ Nuclear and Cytosolic Extraction Reagents (Thermo Scientific) according to the manufacturer’s instructions.

ChIP analyses

ChIP analyses were performed as described previously by Saint-André et al. (45). Antibodies were rabbit IgG, anti-ERα, and anti-Pol II (N-20) (Santa Cruz Biotechnology). Immunoprecipitated DNA and 10% of chromatin input were analyzed by qPCR using the following primers: ERE, forward: 5’-GACCTGTTTGTACACTGACCT-3’; ERE, reverse: 5’-AAAGACGGAGATGAAATATTGACG-3’; 3F1, forward: 5’-TATCAGGCATGTGCTAAGAGC-3’, 3F1, reverse: 5’-GTGTCACCTTGGAGATGTCC-3’, 3F2, forward: 5’-CCTCCAGCTCTCTTCTCAAAAGC-3’, 3F2, reverse: 5’-CCTGAATCTGCACCTTGGG-3’, 3F3, forward: 5’-TTTACCGGAACTACTTAGCC-3’, 3F3, reverse: 5’-TGTATGCTGTTGACTATTCCTCTC-3’, TFF, forward: 5’-CCGGCCATCTTCTCACTTGA-3’, and TFF, reverse: 5’-CCTCCCGCCAGGGTAAATAC-3’. Percentage of input chromatin enriched by each antibody was calculated, and ERαs and Pol II values were normalized by control IgG.
Luciferase reporter assays

Cells expressing Renilla–HCV IRES–Firefly reporter mRNA were treated as indicated in figure legends. Cells were lysed with 1× Passive Buffer, and Renilla and Firefly Luciferase activities were determined using Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer’s protocol. Assays were performed in triplicates and results were analyzed as described in figure legends.

Quantitative RT-PCR

RNA was purified using PureLink™ RNA Mini Kit (Invitrogen) and 0.5 μg of RNA were reverse transcribed into cDNA using iScript cDNA Synthesis Kit (Bio-Rad). For qPCR, cDNA was amplified with iQ™SYBR Green Supermix (Bio-Rad) in a CFX96™ Real-Time PCR System (Bio-Rad). Primers used were TFF1, forward: 5’-ATCTGGGACTAATCCCGTGCTG-3’, eIF3f, forward: 5’-TACAGTGAATAACGGTAC-3’, eIF3f, reverse: 5’-GTCACTTGAGATGCTCAATTAC-3’, and GAPDH, forward: 5’-ATACACCATCTCCAGGACCGA-3’, GAPDH, reverse: 5’-CCTCTCCATGTGGTGAAAGAC-3’.

Cell proliferation assays

Cell viability was determined using MTT Cell Viability Assay Kit from Biotium. Briefly, MCF7 cells were seeded in duplicate into 96-well plates at 5,000 cells/well. Cells were cultured in phenol red–free DMEM supplemented with 5% charcoal-stripped FBS and estrogen (100 nM) for 0, 2, and 4 days. Cell viability was determined according to the manufacturer’s protocol. Absorbance was measured at 570 nm.

Apoptosis assays

HA-expressing MCF7 cells were treated with vehicle (DMSO) or etoposide (50 μM) for 24 h. Selected HA- and HA-eIF3f–expressing cells were treated with vehicle for 24 h. Cells were labeled with Guava Nexin Reagent (EMD Millipore) according to the manufacturer’s protocol. Cells were sorted using a Guava easyCyte Flow Cytometer (EMD Millipore) and analyzed with GuavaSoft software (EMD Millipore).

Statistical analysis

Statistical analysis was performed using the Prism GraphPad 7.0 software. Significance was determined by paired two-tailed Student’s t test. p values < 0.05 were considered significant.

Acknowledgments—We thank Maria Vera for reagents.

References

1. Miller, T. W., Balko, J. M., and Arteaga, C. L. (2011) Phosphatidylinositol 3-kinase and antiestrogen resistance in breast cancer. J. Clin. Oncol. 29, 4452–4461 CrossRef Medline
2. De Marchi, T., Foekens, J. A., Umar, A., and Martens, J. W. (2016) Endocrine therapy resistance in estrogen receptor (ER)-positive breast cancer. Drug Discov. Today 21, 1181–1188 CrossRef Medline
3. Miller, W. R., and Larionov, A. A. (2012) Understanding the mechanisms of aromatase inhibitor resistance. Breast Cancer Res. 14, 201 CrossRef Medline
4. Rondón-Lagos, M., Villegas, V. E., Rangel, N., Sánchez, M. C., and Zarifopoulos, P. G. (2016) Tamoxifen resistance: Emerging molecular targets. Int. J. Mol. Sci. 17, 1357 CrossRef Medline
5. Carroll, J. S., Meyer, C. A., Song, J., Li, W., Geistlinger, T. R., Eeckhoute, J., Brodsky, A. S., Keeton, E. K., Furtk, K. C., Hall, G. F., Wang, Q., Bekirano, S., Sementchekno, V., Fox, E. A., Silver, P. A., Gingeras, T. R., Liu, X. S., and Brown, M. (2006) Genome-wide analysis of estrogen receptor binding sites. Nat. Genet. 38, 1289–1297 CrossRef Medline
6. Nishidate, T., Katagiri, T., Lin, M. L., Mao, Y., Miki, Y., Kasumi, F., Yoshimoto, M., Tsunoda, T., Hirata, K., and Nakamura, Y. (2004) Genome-wide gene-expression profiles of breast-cancer cells purified with laser microbeam microdissection: Identification of genes associated with progression and metastasis. Int. J. Oncol. 25, 797–819 CrossRef Medline
7. Levin, E. R., and Hammes, S. R. (2016) Nuclear receptors outside the nucleus: Extraneuronal signalling by steroid receptors. Nat. Rev. Mol. Cell Biol. 17, 783–797 CrossRef Medline
8. Hershey, J. W., Sonenberg, N., and Mathews, M. B. (2012) Principles of translational control: An overview. Cold Spring Harb. Perspect. Biol. 4, a011528 CrossRef Medline
9. Himnebusch, A. G. (2014) The scanning mechanism of eukaryotic translation initiation. Annu. Rev. Biochem. 83, 779–812 CrossRef Medline
10. Gao, B., and Roux, P. P. (2015) Translational control by oncogenic signaling pathways. Biochim. Biophys. Acta 1849, 753–765 CrossRef Medline
11. Holz, M. K., Ballif, B. A., Gygi, S. P., and Blenis, J. (2005) mTOR and 56Kl mediate assembly of the translation preinitiation complex through dynamic protein interaction and ordered phosphorylation events. Cell 123, 569–580 CrossRef Medline
12. Chu, J., Cargnello, M., Topisirovic, I., and Pelletier, J. (2016) Translation initiation factors: Reprogramming protein synthesis in cancer. Trends Cell Biol. 26, 918–933 CrossRef Medline
13. Truitt, M. L., and Ruggero, D. (2016) New frontiers in translational control of the cancer genome. Nat. Rev. Cancer 16, 288–304 CrossRef Medline
14. vaklavas, C., Blume, S. W., and Grizzle, W. E. (2017) Translational dysregulation in cancer: Molecular insights and potential clinical applications in biomarker development. Front. Oncol. 7, 158 CrossRef Medline
15. de la Parra, C., Walters, B. A., Geter, P., and Schneider, R. J. (2018) Translation initiation factors and their relevance in cancer. Curr. Opin. Genet. Dev. 48, 82–88 CrossRef Medline
16. Bhat, M., Robichaud, N., Hulea, L., Sonenberg, N., Pelletier, J., and Topisirovic, I. (2015) Targeting the translation machinery in cancer. Nat. Rev. Drug Discov. 14, 261–278 CrossRef Medline
17. Chu, J., and Pelletier, J. (2015) Targeting the eIF4A RNA helicase as an anti-neoplastic approach. Biochim. Biophys. Acta 1849, 781–791 CrossRef Medline
18. Lu, C., Makala, L., Wu, D., and Cai, Y. (2016) Targeting translation: eIF4E as an emerging anticancer drug target. Expert Rev. Mol. Med. 18, e2 CrossRef Medline
19. Chu, J., and Pelletier, J. (2018) Therapeutic opportunities in eukaryotic translation. Cold Spring Harb. Perspect. Biol. 10, a032995 CrossRef Medline
20. valašek, L. S., Zeman, J., Wagner, S., Beznosková, P., Pavliková, Z., Mohammad, M. P., Hronová, V., Herrmannová, A., Hashem, Y., and Gunisová, S. (2017) Embraced by eIF3: Structural and functional insights into the roles of eIF3 across the translation cycle. Nucleic Acids Res. 45, 10948–10968 CrossRef Medline
21. Hershey, J. W. (2015) The role of eIF3 and its individual subunits in cancer. Biochim. Biophys. Acta 1849, 792–800 CrossRef Medline
22. Zhang, L., Pan, X., and Hershey, J. W. (2007) Individual overexpression of five subunits of human translation initiation factor eIF3 promotes malignant transformation of immortal fibroblast cells. J. Biol. Chem. 282, 5790–5800 CrossRef Medline
ERα regulates expression of eIF3f

23. Wagner, S., Herrmannová, A., Šikrová, D., and Valášek, L. S. (2016) Human eIF3b and eIF3a serve as the nucleation core for the assembly of eIF3 into two interconnected modules: The yeast-like core and the octamer. *Nucleic Acids Res.* **44**, 10772–10788 CrossRef Medline

24. Lee, A. S., Kranzusch, P. J., and Cate, J. H. (2015) eIF3 targets cell-proliferation messenger RNAs for translational activation or repression. *Nature* **522**, 111–114 CrossRef Medline

25. Lee, A. S., Kranzusch, P. J., Doudna, J. A., and Cate, J. H. (2016) eIF3d is an mRNA cap-binding protein that is required for specialized translation initiation. *Nature* **536**, 96–99 CrossRef Medline

26. Zhao, W., Li, X., Wang, J., Wang, C., Jia, Y., Yuan, S., Huang, Y., Shi, Y., and Tong, Z. (2017) Decreasing eukaryotic initiation factor 3C (eIF3C) suppresses proliferation and stimulates apoptosis in breast cancer cell lines through mammalian target of rapamycin (mTOR) pathway. *Med. Sci. Monit.* **23**, 4182–4191 CrossRef Medline

27. Shi, J., Kahle, A., Hershey, J. W., Honchak, B. M., Warnke, J. A., Leong, S. P., and Nelson, M. A. (2006) Decreased expression of eukaryotic initiation factor 3f deregulates translation and apoptosis in tumor cells. *Oncogene* **25**, 4923–4936 CrossRef Medline

28. Guertin, M. I., Zhang, X., Coomrod, S. A., and Hager, G. L. (2014) Transient estrogen receptor binding and p300 redistribution support a squelching mechanism for estradiol-repressed genes. *Mol. Endocrinol.* **28**, 1522–1533 CrossRef Medline

29. Harris, T. E., Chi, A., Shabanowitz, J., Hunt, D. F., Rhoads, R. E., and Lawrence, J. C., Jr. (2006) mTOR-dependent stimulation of the association of eIF4G and eIF3 by insulin. *EMBO J.* **25**, 1659–1668 CrossRef Medline

30. Wendel, H. G., Silva, R. L., Malina, A., Mills, J. R., Zhu, H., Ueda, T., Watanabe-Fukunaga, R., Fukunaga, R., Teruya-Feldstein, J., Pelletier, J., and Lowe, S. W. (2007) Dissecting eIF4 action in tumorigenesis. *Genes Dev.* **21**, 3232–3237 CrossRef Medline

31. Echeverria, P. C., and Picard, D. (2010) Molecular chaperones, essential partners of steroid hormone receptors for activity and mobility. *Biochim. Biophys. Acta* **1803**, 641–649 CrossRef Medline

32. Liang, J., and Shang, Y. (2013) Estrogen and cancer. *Annu. Rev. Physiol.* **75**, 225–240 CrossRef Medline

33. Altucci, L., Addeo, R., Cicatiello, L., Dauvois, S., Parker, M. G., Truss, M., Beato, M., Sica, V., Bresciani, F., and Weisz, A. (1996) 17β-Estradiol induces cyclin D1 gene transcription, p36D1-p34cdc4 complex activation and p105rb phosphorylation during mitogenic stimulation of G(1)-arrested human breast cancer cells. *Oncogene* **12**, 2315–2324 Medline

34. Wagner, S., Herrmannová, A., Malik, R., Peclinovská, L., and Valášek, L. S. (2014) Functional and biochemical characterization of human eukaryotic translation initiation factor 3 in living cells. *Mol. Cell. Biol.* **34**, 3041–3052 CrossRef Medline

35. Zhang, L., Smit-McBride, Z., Pan, X., Rheinhardt, J., and Hershey, J. W. (2008) An oncogenic role for the phosphorylated h-subunit of human translation initiation factor eIF3. *J. Biol. Chem.* **283**, 24047–24060 CrossRef Medline

36. Villa, N., Do, A., Hershey, J. W., and Fraser, C. S. (2013) Human eukaryotic initiation factor 4G (eIF4G) protein binds to eIF3c, -d, and -e to promote mRNA recruitment to the ribosome. *J. Biol. Chem.* **288**, 32932–32940 CrossRef Medline

37. Wen, F., Zhou, R., Shen, A., Choi, A., Uribe, D., and Shi, J. (2012) The tumor suppressive role of eIF3f and its function in translation inhibition and rRNA degradation. *PLoS One* **7**, e34194 CrossRef Medline

38. Shi, J., Hershey, J. W., and Nelson, M. A. (2009) Phosphorylation of the eukaryotic initiation factor 3f by cyclin-dependent kinase 11 during apoptosis. *FEBS Lett.* **583**, 971–977 CrossRef Medline

39. Musgrove, E. A., Sergio, C. M., Loi, S., Inman, C. K., Anderson, L. R., Alles, M. C., Pinese, M., Caldon, C. E., Schütte, J., Gardiner-Garden, M., Ormandy, C. J., McArthur, G., Butt, A. J., and Sutherland, R. L. (2008) Identification of functional networks of estrogen- and c-Myc-responsive genes and their relationship to response to tamoxifen therapy in breast cancer. *PLoS ONE* **3**, e2987 CrossRef Medline

40. Cuesta, R., and Holz, M. K. (2016) RSK-mediated down-regulation of PDCD4 is required for proliferation, survival, and migration in a model of triple-negative breast cancer. *Oncotarget* **7**, 27567–27583 CrossRef Medline

41. Alayev, A., Salamon, R. S., Manns, S., Schwartz, N. S., Berman, A. Y., and Holz, M. K. (2016) Estrogen induces RAD51C expression and localization to sites of DNA damage. *Cell Cycle* **15**, 3230–3239 CrossRef Medline

42. Maruani, D. M., Spiegel, T. N., Harris, E. N., Shachter, A. S., Unger, H. A., Herrero- González, S., and Holz, M. K. (2012) Estrogenic regulation of S6K1 expression creates a positive regulatory loop in control of breast cancer cell proliferation. *Oncogene* **31**, 5073–5080 CrossRef Medline

43. Krüger, M., Beger, C., Welch, P. J., Barber, J. R., Manns, M. P., and Wong-Staal, F. (2001) Involvement of proteasome α-subunit PSMA7 in hepatitis C virus internal ribosome entry site-mediated translation. *Mol. Cell. Biol.* **21**, 8357–8364 CrossRef Medline

44. Saint-André, V., Batsché, E., Rachez, C., and Muchardt, C. (2011) Histone H3 lysine 9 trimethylation and HP1 γ favor inclusion of alternative exons. *Nat. Struct. Mol. Biol.* **18**, 337–344 CrossRef Medline