Protein Phosphatase 2A Regulates Estrogen Receptor α (ER) Expression through Modulation of ER mRNA Stability*

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Protein phosphatase 2A (PP2A) is a ubiquitously expressed member of the serine-threonine phosphatase family that is involved in regulation of many cellular processes including transcription, translation, cellular metabolism, and apoptosis. Because of a correlation between PP2A and estrogen receptor α (ER) expression in several human breast cancer cell lines, the effect of PP2A on regulation of ER expression in the human breast cancer cell line MCF-7 was studied. Inhibition of PP2A using the pharmacologic inhibitor okadaic acid at 250 nM for 16 h resulted in a 60% reduction in PP2A activity in MCF-7 cells concurrent with a 75% reduction in ER mRNA and protein expression. Similar results were obtained with a small interfering RNA probe that specifically inhibited PP2A expression. ER promoter studies showed that regulation of ER through the PP2A pathway did not occur through transcriptional activation. Rather, PP2A mediated ER expression through modulation of ER mRNA stability through degradation of ER mRNA, reversible with concomitant treatment with the proteasomal inhibitor MG 132. These data suggest a novel pathway controlling ER expression resulting from the activation of PP2A, potentially providing a novel therapeutic target.

Protein phosphatase 2A (PP2A)§ is a ubiquitously expressed member of a large protein phosphatase family involved in the regulation of cell proliferation, cell differentiation, RNA transcription, DNA repair, and apoptosis (1–4). PP2A has been shown to inhibit major signal transduction pathways, including the phosphatidylinositol 3-kinase/AKT and mitogen-activated protein kinases pathways. Decreased PP2A activity either through pharmacologic inhibition or RNA interference techniques results in increased apoptosis and inhibition of gene expression, suggesting an important role for PP2A in the regulation of cell growth (1, 5–11). PP2A also contributes to the control of epigenetic gene regulation by decreasing histone protein phosphorylation and increasing histone acetylation (12–14).

PP2A is a holoenzyme comprised of three subunits, a structural subunit (A), a regulatory subunit (B), and a catalytic subunit (C). A and C subunits are highly conserved, each consisting of two highly homologous isoforms, α and β, whereas the B subunit is much more complex, containing three distinct families, B’, B’, and B”, each with several homologous members (3). Considering all the potential interactions between the multitudes of subunits, 75 different combinations of A-B-C subunits could form, providing the potential for immense diversity and target specificity within the cell.

Dysregulation of PP2A has been shown to be a contributing factor in many cancer types. Mutations in the A subunit, resulting in decreased interaction with the B and C subunits and improper formation of the holoenzyme, have been noted in a subset of colorectal and breast cancers (15, 16). In addition, overexpression of PP2A alone resulted in cellular transformation in human embryonic kidney cells (17). Aberrant subcellular localization of PP2A has been shown to alter cellular growth and apoptosis in hepatic cancer cells (18). These studies suggest that changes in PP2A can contribute directly to cancer development, increased cellular growth, and decreased apoptosis (17, 18). Decreased nuclear localization of PP2A is correlated with decreased PP2A activity in estrogen receptor α (ER)-negative compared with ER-positive human breast cancer cell lines, suggesting that PP2A could be a contributing factor to the hormone-independent phenotype that comprises a large subset of all human breast cancer cases (19). The mechanism of PP2A-mediated ER expression is still largely unknown.

Approximately 70–80% of all breast tumors express ER protein. These tumors tend to grow more slowly and are associated with a slightly better prognosis (20). More importantly, the detection of ER in breast carcinoma cells is an important indicator of potential response to endocrine therapy as tumors expressing ER protein are the most likely to respond to endocrine therapy, whereas those lacking ER seldom respond (21). The molecular mechanisms controlling these effects are still being defined. Determining the molecular mechanisms underlying ER expression, therefore, is crucial to the understanding and treatment of breast cancer.

ER expression is controlled at the transcriptional level through both epigenetic mechanisms and activation of the proximal (P1) ER promoter (22–25). ER expression is also regulated at the post-transcriptional level through modulation of mRNA stability via interactions with the 3’-UTR (26–28). Previous data from our laboratory suggest that the Bβ subunit of PP2A is re-expressed coordinately with ER when ER-negative cells are treated with epigenetic modifiers (29). These data suggest that PP2A expression is upstream of ER expression.
and may play an important role in the transcriptional control of ER (29).

### MATERIALS AND METHODS

**Cell Culture, Maintenance, and Treatment**—MDA-MB-231, MDA-MB-468, T47D, and MCF-7 cells were cultured at 37°C, 5% CO2 in Dulbecco’s modified Eagle’s medium supplemented with 5% fetal bovine serum (HyClone, Logan, UT), and 2 μM l-allyl-l-glutamate (Meditech, Herndon, VA). For MDA-MB-231 ER+ (231ER+) cells, the entire coding region of ER was PCR amplified using MCF-7 cDNA as template and cloned into the expression vector pIREShyg2 (Clontech, Palo Alto, CA). The cloned cDNA was sequenced to verify the ER cDNA sequence. The construct was then transfected into MDA-MB-231 cells using Lipofectamine according to the manufacturer’s protocol (Invitrogen). Stable colonies were selected using Hygromycin B (Calbiochem, La Jolla, CA) at a concentration of 800 μg/ml in Dulbecco’s modified Eagle’s medium. Stable clones (termed 231ER+) were maintained in selection medium to ensure high levels of ER expression. Unless otherwise indicated, MDA-MB-231 and MCF-7 cells were plated at 300,000 cells/10-cm plate and 500,000 cells/10-cm plate, respectively, 24 h before treatment with okadaic acid (250 nM, OA) (Calbiochem) for 16 h.

**Reverse, PCR, and Real-Time PCR**—RNA was harvested using the TRIzol reagent (Invitrogen) as previously described (30). cDNA was synthesized from 3 μg total RNA using MMLV reverse transcriptase (Invitrogen) and oligo(dT) primers (Invitrogen) at 37°C for 1 h. Conventional PCR was performed in cDNA samples as previously described (30) using the following primers: ERα S, GCC CCC AGT AGT TCG AA; AS, TGG CAT AAG TGG TGC ATG AT (55°C, 35 cycles). cDNA was amplified using SYBR green (Sigma) for ER, PP2A, and GAPDH at an annealing temperature of 60°C with 1-min annealing time (245 cycles). GAPDH was used as an internal control to normalize samples. Experiments were performed three times, and each measurement was taken in duplicate.

**Western Blotting**—MCF-7, T47D, MDA-MB-231, and MDA-MB-468 were transfected with Opti-MEM reduced serum medium (Invitrogen) for 5 min at room temperature. Specific PP2A-ε siRNA probes (5 nM) or nontargeting DNA probes (5 nM) (Dharmacon) were added to the Oligofectamine mixture and incubated at room temperature for 10 min for proper transfection complex formation. Transfection efficiency was then determined by incubating cells in serum-free medium. After 4 h, serum concentrations in each well were adjusted to 5%, and cells were harvested after 72 h for RNA and protein.

**Promoter Deletion and Luciferase Assay**—MCF-7 cells were seeded at 2 × 10^4 cells/well in 24-well plates 24 h prior to transfection. 3 μg of Gene Jammer (Invitrogen) transfection reagent was used to transiently transfected 0.5 μg of pERPlux pGLO basic luciferase ER promoter deletion construct (−2769, −1000, −745, or −245 to +212) (kindly provided by Dr. Suzanne Fuqua, Baylor College of Medicine). 1 μg of β-galactosidase expression vector was cotransfected with all plasmids to determine transfection efficiency. At 48 h, luciferase activity was measured on a Monolight luminometer using the BrightGlo luciferase assay kit (Promega), and β-galactosidase activity was determined using the β-galactosidase activity kit (Promega). Experiments were completed at three times, and each measurement was taken in duplicate.

**Electrophoretic Mobility Shift Assay**—Nuclear proteins were harvested from MCF-7 cells with or without okadaic acid treatment for 16 h at 250 nM as previously described (31). Nuclear extract was incubated with 8% glycerol and either poly(dI-dC) or poly(dG-dC) (0.2 μg) (Pharmacia) as indicated for 20 min on ice. γ−32P-endabeled 5′-ACCTAGACAGCTCTGGTCAATTCCACAAGCTCCTGGCCTCTAGAACTGCACCAGAAAGATGGTGATATCGCCACTTATCGTCCTCTCCAC; P2A 5′-TAT TAC TCT AGT AGA ATT CAA CCA CCC GGG 3′, 5′-GAA GGT GAA GGT CGG AGT C 3′, 5′-GAA GAT GAG GTT AGC TC 3′. Real-time PCR data were acquired and analyzed using Sequence Detector v1.7 software (PerkinElmer) and normalized using GAPDH housekeeping gene detection.

**PP2A Activity Assay**—PP2A activity in whole cell MCF-7 lysates was determined by the PP2A serine/threonine phosphatase assay according to the manufacturer’s protocol (Upstate Biotechnology, Charlottesville, VA). Briefly, PP2A c subunit was immunoprecipitated from 200 μg of nuclear extracts using 2 μg of anti-PP2Ac antibody (Upstate clone 1D6) for 2 h at 4°C. Lysates were extensively washed in Tris-buffered saline and Ser-Thr phosphatase assay buffer. Phosphatase activity was determined by conversion of ppnpp, nPPNP, added dihydroxyacetone phosphate, and the beads for 15 min at 37°C. Accumulation of dephosphorylated substrate was measured in the supernatant using a spectrophotometer (Molecular Devices, Sunnyvale, CA) at 405-nm wavelength.

**Western Blotting**—MCF-7, T47D, MDA-MB-231, and MDA-MB-231 ER+ cells were treated with 250 nM OA for 16 h. Protein was harvested from cells using whole cell lysis buffer (150 mM NaCl, 10 mM Tris-HCl, 5 μM EDTA, 0.1% Triton X-100, 5% glycerol) on ice for 30 min. Equal concentrations of whole cell lysates were denatured in 2× Laemmli’s sample buffer and separated on 12% polyacrylamide gels (GenMate; ISC Biotechnology, Kaysville, UT). Separated proteins were transferred to polyvinylidene difluoride membrane (Bio-Rad) at 100 volts for 1.5–2 h. Nonspecific antibody binding was blocked with 5% milk in phosphate-buffered saline for 1 h at room temperature. Membranes were then hybridized with primary antibody (ER, H20, Santa Cruz, 1:500; PP2A, H300, Santa Cruz, 1:500; PP2AB, 05.592, Upstate, 1:1000; PP2AB’ 07.334, Upstate, 1:1000; PP2AC 1D6, Upstate, 1:1000; Actin, Sigma, 1:10,000) overnight at 4°C while gently rocking before incubation with the appropriate secondary antibody (DAKO, 1:2000) for 1 h at room temperature. Proteins were detected by chemiluminescence (ECL Western Blotting Reagents).

**RNA Interference**—MCF-7 cells were seeded at 70,000 cells/well in 6-well plates and allowed to adhere for 24 h. Cells were then transiently transfected with specific PP2Aε subunit and non-targeting DNA control probes (Dharmacon, Lafayette, CO) using Oligofectamine transfection reagent (Qiagen, Valencia, CA) according to the manufacturer’s protocol. Briefly, Oligofectamine reagent (200 μl) was incubated with Opti-MEM reduced serum medium (Invitrogen) for 5 min at room temperature.

**RESULTS**

**PP2A Subunits Are Abundantly Expressed in Human Breast Cancer Cell Lines and Are Inhibited by Okadaic Acid Treatment**—Western blotting of whole cell lysates from MCF-7, T47D, MDA-MB-231, and MDA-MB-468 cells showed that PP2A A, B, B′, and C subunits are abundantly expressed in several human breast cancer cells of variable phenotype (Fig. 1). OA is a widely used PP2A inhibitor, known to inhibit PP2A activity at nM concentrations through blockade of the catalytic subunit (PP2Ac). In MCF-7 cells, OA treatment had no effect on PP2A mRNA expression as determined by real-time PCR (Fig. 2A) but significantly decreased PP2A activity (Fig. 2B) (p < 0.01).

**Inhibition of PP2A Decreased ER mRNA, Protein, and Activity**—Previous reports from our laboratory (29) and others (19) have suggested a role for PP2A in the regulation of ER. Therefore, the effect of OA on ER expression was examined in ER-positive MCF-7 cells. OA inhibition of PP2A resulted in an 80% reduction in ER mRNA expression as determined by real-time PCR (Fig. 3A) (p < 0.001). This decrease in ER mRNA translated into a significant decrease in ER protein (Fig. 3C). The decrease in ER was functionally significant as demonstrated by...
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FIG. 1. Expression of PP2A subunits is ubiquitous and uniform. Expression of four PP2A subunits, A, B, B', and C was determined by Western blotting in whole cell lysates from ER-positive MCF-7 and T47D and ER-negative MDA-MB-231 and MDA-MB-468 cells.

FIG. 2. OA treatment of MCF-7 cells results in decreased PP2A activity without alteration in PP2A mRNA expression. MCF-7 cells were treated with or without 250 nM OA for 16 h. A, expression of PP2Ac subunit mRNA following OA treatment was quantitated by real-time PCR and normalized to GAPDH expression. B, PP2A activity was measured in MCF-7 whole cell lysates with or without OA treatment. Shown is the mean ± S.E. of three separate experiments conducted in duplicate. *, p < 0.01 as determined by Student’s t test.

decreased mRNA and protein expression of the progesterone receptor (PR), a well recognized ER-responsive gene (Fig. 3, B and C) (p < 0.001). In contrast, OA treatment had no effect on protein expression of another member of the nuclear receptor family, the vitamin D receptor (VDR) (Fig. 3C), which unlike PR is not regulated by ER activity.

Genetic Inhibition of PP2A with Small Interfering RNAs Resulted in a Significant Decrease in ER Expression—Although the pharmacologic inhibitor OA has been shown to be 100–1000-fold more specific for PP2A than for any other protein phosphatase family member, the effects of PP2A inhibition were confirmed using a genetic approach, small interfering RNAs (siRNA) targeting PP2Ac. Transfection of MCF-7 cells with siRNA against PP2Ac resulted in a significant decrease in PP2A mRNA and protein expression (Fig. 4, A and D) (p < 0.05). This inhibition of PP2A resulted in a significant decrease in ER mRNA and protein (Fig. 4, B and D) (p < 0.05). Inhibition also resulted in a decrease in ER activity as determined by a decrease in mRNA and protein expression of the ER-responsive gene, PR (Fig. 4, C and D) (p < 0.01). As with OA treatment, siRNA inhibition of PP2A had no effect on VDR protein expression, confirming the specificity of the effect of PP2A on the ER pathway (Fig. 4D).

PP2A Does Not Alter ER Promoter Activity—ER expression is regulated at both the transcriptional and post-transcriptional levels. To test whether PP2A exerts its effects on ER promoter activation, the effect of OA treatment on ER transcription was tested using MCF-7 cells transiently transfected with pERpGL3 basic luciferase promoter constructs (Fig. 5A) (22). de Graffenried et al. (22) have reported that the ER promoter region is constitutively active in MCF-7 cells and that the most proximal 245 base pairs upstream of the transcriptional start site contains a SP1 binding site that is necessary and sufficient for activation of the ER promoter. Analysis of ~3000 base pairs of the ER promoter (~2700 to +212) in MCF-7 cells showed substantial ER promoter activity that is not altered with OA treatment (Fig. 5B). Although there appears to be a decrease in ER promoter activity with OA treatment of the ~1000 construct, neither the full-length promoter ~2700 construct nor the proximal ~245 region that contains known transcription factor binding sites showed any difference in promoter activation between control and OA-treated MCF-7 cells (Fig. 5B).

Studies have shown binding of transcription factors, including SP1 and USF-1, to the ~245 region of the ER promoter transactivates ER expression (22, 23). Evaluation of transcription factor binding to the ~245 construct via electrophoretic mobility shift assay analysis did not show changes in transcription factor binding in OA-treated MCF-7 nuclear extracts compared with control, consistent with our findings using the luciferase promoter constructs (Fig. 5C).

PP2A Activates ER through Modulation of mRNA Stability—Because OA treatment led to a decrease in ER mRNA by reverse transcription-PCR and real-time PCR without a difference in promoter activation or transcription factor binding, the role of the 3'-UTR in the regulation of ER expression was assessed using the 231ER+ cell model. These cells were generated by stable transfection of an expression construct of ER cDNA cloned from MCF-7 cells under the control of a cytomegalovirus promoter. 231ER+ cells express high levels of functional ER localized in the nucleus (Fig. 6 and data not shown). As the expression construct contains only the coding sequence of ER without the 3'-UTR, any mRNA-stabilizing effects of the 3'-UTR are absent in 231ER+ cells. Surprisingly, treatment of 231ER+ cells with OA resulted in a significant increase in ER protein expression (Fig. 6); similar results were obtained with MCF10A cells that were transfected with the same ER construct (data not shown). This enhanced ER expression stands in marked contrast with the profound decrease in ER expression following OA treatment of the innately ER-positive MCF-7 and T47D cells. To verify that decreases in ER expression detected in MCF-7 cells reflected changes in endogenous expression of ER, a second ER-positive cell line, T47D, was studied after treatment with OA (10 nM). As seen following PP2A inhibition in MCF-7 cells, ER protein expression in T47D cells was significantly inhibited to below the level of detection by Western blotting (Fig. 6).

PP2A Stabilizes ER mRNA through the 3'-UTR—Because OA treatment of MCF-7 cells did not alter promoter activity or
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FIG. 4. siRNA interference of PP2A RNA expression results in decreased ER expression and activity. MCF-7 cells were transiently transfected with siRNA probes specific for PP2A or nonsense DNA sequences for 72 h, and RNA and protein were isolated. mRNA expression was determined from RNA harvested from these cells by real-time PCR and normalized to GAPDH mRNA expression. siRNA inhibition of PP2A resulted in significant inhibition of mRNA expression of PP2A (p < 0.05 as determined by Student’s t test) (A), ER (p < 0.05 determined by Student’s t test) (B), and PR expression (p < 0.01 determined by Student’s t test) (C) compared with transfection of a nonsense DNA probe. Shown is the mean ± S.E. of three experiments conducted in duplicate. D, PP2A inhibition resulted in decreased PP2A, ER, and PR protein expression by Western blotting of MCF-7 whole cell lysates. There was no change in VDR expression following PP2A inhibition. Actin expression was used as a loading control.

transcription factor binding (Fig. 5, A and B), the contribution of the 3′-UTR to ER mRNA stabilization was evaluated. MCF-7 and 231ER+ cells were treated with the transcriptional inhibitor actinomycin D (4 μg/ml) in the presence or absence of OA (250 nM) for 0, 2, or 8 h. ER mRNA levels were then quantified by real-time PCR. The percent of mRNA remaining after actinomycin D inhibition of transcription was significantly reduced in MCF-7 cells treated with OA and actinomycin D compared with actinomycin D alone. ER mRNA half-life was significantly reduced from 4.5 to ~1.5 h (Fig. 7A) (p < 0.006). As expected, no change in ER mRNA decay or half-life was detected in 231ER+ cells (that lack the ER 3′-UTR) in the presence or absence of OA (Fig. 7B).

We next tested whether inhibition of the proteasome using the specific inhibitor MG132 would block OA-mediated ER mRNA degradation, suggesting that PP2A-mediated control of ER expression occurs through protein interactions with the ER 3′-UTR. MDA-MB-231 cells were transiently transfected with the ER 3′-UTR construct containing 3.7 kb of the 3′-UTR and treated for 4 h with OA (250 nM), MG132 (10 μM), or a combination of OA and MG132. Actinomycin D (4 μg/ml) was then added to inhibit further mRNA synthesis. The percent of ER mRNA remaining was measured by real-time PCR and normalized to GAPDH mRNA expression. Inhibition of the proteasomal pathway prevented OA-mediated ER mRNA degradation (Fig. 8). Similar studies using the Hsp70 inhibitor KNK437 (Calbiochem) to ascertain whether the Hsp70 chaperone protein might play a role in OA-mediated ER mRNA degradation showed no effect (data not shown). Together these results indicate that PP2A stabilizes ER mRNA through interactions with the 3′-UTR and provide a link between PP2A activation and proteasomal degradation.

DISCUSSION

ER expression is regulated at multiple levels in human breast cancer cell lines. These include transcription factor-promoter interactions at the transcriptional level, phosphorylation at the post-transcriptional level, and degradation through altered mRNA stability and proteasomal degradation (22, 23, 26–28). ER expression can also be silenced through epigenetic mechanisms in some ER-negative human breast cancer tumors (24, 25, 30, 32, 33). All of these mechanisms appear to be interrelated and tightly control the regulation of ER expression in the cell. MCF-7 cells are the best characterized model of ER-expressing human breast cancer cells. One mechanism controlling ER expression in these cells is through activation of the promoter region and interaction of transcription factors with the proximal promoter (22, 23). Studies have also shown that ER contains a long 3′-UTR that contains numerous AUUA sequences and binding sites that have been shown to affect mRNA stability and contribute to regulation of ER gene expression in MCF-7 cells (27, 28).

Several studies have implicated PP2A in the regulation of ER expression. Gopalakrishna et al. (19) first reported a correlation between PP2A activity and ER expression in a panel of human breast cancer cell lines. Subsequent studies showed that PP2A activation results in inhibition of the mitogen-activated protein kinase pathway (extracellular signal-regulated kinase), resulting in decreased ER phosphorylation and activity without an alteration of ER protein expression (7, 8, 34, 35). More recently, Lu et al. (36) reported that PP2A inhibition increases ER expression in rat pulmonary vein endothelial cells infected with an adenoviral ER-green fluorescence protein (GFP) construct, Rad91 adeno-GFP-ER. Our previous data suggest a role for PP2A in mediating ER expression (29). In a microarray analysis of ER-negative MDA-MB-231 cells treated with epigenetic modulators that induce ER expression, up-regulation of PP2A expression was detected in conjunction with ER, suggesting that increased PP2A expression may play a role in the regulation of ER expression (29). Based on these findings, we sought to further characterize the role of PP2A in ER expression in MCF-7 cells.

Inhibition of PP2A activity, either through pharmacologic inhibition with OA or by genetic knockdown by siRNA, resulted in a significant decrease in ER mRNA and protein expression (Figs. 3 and 4) and diminished ER activity as determined by down-regulation of an ER-responsive gene, PR. This PP2A-mediated gene expression control is specific for the ER pathway in that no change in expression of another member of the nuclear steroid receptor family, VDR, was detected using either method (Figs. 3C and 4D).

Although ER expression is regulated through transcriptional mechanisms, PP2A-mediated ER expression does not involve increased promoter activation or transcription factor interactions (Fig. 5). Transcriptional regulation of ER requires binding of transcription factors including SP1 and USF1 to the proximal promoter region (37). PP2A has been shown to physically interact with SP1 in T lymphocytes, suggesting that a potential mechanism of PP2A-mediated ER expression could result from changes in binding of SP1 and activation of ER transcription (38). However, no change in ER promoter activation using
Luciferase ER promoter constructs or transcription factor binding to the ER P1 promoter was detected, thereby effectively eliminating transcriptional control as a mechanism of PP2A-mediated ER expression in MCF-7 cells (Fig. 5, B and C).

Unlike in MCF-7 or T47D cells, OA treatment of 231ER/H11001 cells gave the seemingly paradoxical result of increased ER expression (Fig. 6). Similar findings were seen after OA treatment of MCF10A cells engineered to overexpress ER (data not shown) as well as Rad91 cells overexpressing ER cDNA (36).

The overexpressed ER cDNA in the 231ER/H11001 and MCF10AER+/+ cells was cloned from MCF-7 cells and shares an identical coding sequence, but it lacks the endogenous ER 3'-UTR that contains numerous binding sites for AUUA- and other RNA-binding proteins. The importance of this region for ER regulation is supported by the actinomycin and proteasome inhibition studies that show a significant decrease in ER mRNA half-life in OA-treated MCF-7 cells compared with untreated MCF-7 cells and no difference in ER mRNA half-life in 231ER+/+ cells that lack the 3'-UTR (Fig. 7). This OA-mediated ER inhibition was clearly reversed in the presence of the proteasomal inhibitor MG132 (Fig. 8) but not altered in the presence of the Hsp70 chaperone protein inhibitor KNK437 (data not shown). That this 3'-UTR mechanism might be more global is suggested by findings after PP2A inhibition of another breast cancer cell line, MDA-MB-468, that resulted in decreased c-fos mRNA stability through interaction with AUUA sequences in the c-fos 3'-UTR.
determined by Student’s t test). Concomitant treatment with both OA and the proteasomal inhibitor MG132 eliminates the effect of OA on ER mRNA degradation (*, p < 0.05 compared with OA treatment alone, determined by Student’s t test).

3′-UTR, suggesting that PP2A may increase c-fos mRNA stability as well (38). Experiments using the specific proteasomal inhibitor MG132 in conjunction with OA treatment clearly provide a link between PP2A and the proteasome. MG132 treatment reversed the OA-mediated ER mRNA decay, indicating that PP2A regulates ER expression through modulation of the ER 3′-UTR, resulting in proteasomal degradation (Fig. 8), suggesting that ER mRNA decay is regulated by PP2A-mediated binding of a factor to the 3′-UTR. Proteasomal degradation of this protein leads to ER mRNA degradation in the absence of PP2A activity. We, therefore, propose that PP2A-mediated ER mRNA stability depends on the presence of and protein binding to the ER 3′-UTR.

In summary, our data suggest that PP2A plays a significant role in ER gene expression by increasing ER mRNA stability and half-life. Further, the importance of the 3′-UTR region in regulating ER expression is highlighted. Finally, these studies that utilize cell lines that endogenously express ER as a physiologically relevant model suggest that PP2A could be an effective treatment target.

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FIG. 8. OA-mediated ER mRNA destabilization is associated with the proteasome pathway. MDA-MB-231 cells were transiently transfected with the ER 3′-UTR construct. Cells were treated for 4 h with OA (250 nM), MG132 (10 μM), or a combination of OA and MG132. Actinomycin D (4 μg/ml) was then added to block nascent RNA synthesis, and the cells were incubated for 4 more hours under normal culture conditions before harvest for ER mRNA measurement. The percent of ER mRNA remaining was measured by real-time PCR and normalized to GAPDH mRNA levels. Shown is the mean ± S.E. from two experiments, each conducted in duplicate. ER mRNA levels are significantly decreased by OA treatment compared with control (**, p < 0.01 as determined by Student’s t test). Comitant treatment with both OA and the proteasomal inhibitor MG132 eliminates the effect of OA on ER mRNA degradation (+, p < 0.05 compared with OA treatment alone, determined by Student’s t test).