Post-transcriptional Destabilization of Estrogen Receptor mRNA in MCF-7 Cells by 12-O-Tetradecanoylphorbol-13-acetate

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The effect of 12-O-tetradecanoylphorbol-13-acetate (TPA) on the regulation of the estrogen receptor (ER) was investigated in this study. After treatment with 100 nM TPA the concentration of receptor protein was measured using an enzyme immunoassay. By 24 h the receptor protein declined by about 80% from a level of approximately 236 fmol of ER/mg of protein in control cells to 50 fmol of ER/mg of protein in cells treated with TPA. Similar results were obtained with an estrogen receptor ligand binding assay. After removal of TPA, the level of ER returned to control values. 4a-Phorbol, a compound related to TPA, had no effect on ER. The effects of TPA on ER expression appear to be mediated by activation of protein kinase C as H-7, an inhibitor of protein kinase C, blocks these effects. In addition to the effect on ER protein, TPA treatment also resulted in a decrease in the steady-state level of ER mRNA as determined by a RNase protection assay. The metabolic inhibitor cycloheximide was unable to prevent the TPA-induced decrease in ER mRNA. Transcription run-off experiments demonstrated that TPA had no effect on ER gene transcription. A half-life study demonstrated that TPA decreased ER mRNA half-life by a factor of 6 from approximately 4 h in control cells to 40 min in TPA-treated cells. These data suggest that the decline in ER expression is mediated by post-transcriptional destabilization of ER mRNA.

Phorbol esters are tumor promoters that display a wide range of activities in mammalian tissues and cells. They have been shown to produce different and often opposite biological responses in different types of cells. In many cell systems phorbol esters stimulate cell proliferation and inhibit cell differentiation (1, 2). In others they induce growth arrest and differentiation (3, 4). These compounds have been shown to affect cell morphology, phospholipid metabolism, cell communication, and protein phosphorylation (5, 6). Although the precise mechanism of action of phorbol esters remains unknown it is believed that phorbol esters may mediate many of their effects by regulating the activity and expression of protein kinase C (7-9), a high affinity receptor for these compounds. Recently it has been shown that phorbol esters modulate gene transcription. Among the genes whose transcription is induced by TPA1 are c-fos (10), c-myc (10, 11), c-sis (12), collagenase (13), and stromelysin (13, 14). In these genes a common cis element confers induction by phorbol esters in a manner similar to the responsive elements of steroid hormones (15). These cis elements appear to be recognized by trans-acting factors. The activity or abundance of these trans-acting elements is modulated by protein kinase C in response to phorbol ester treatment. Two of these trans-acting factors have been identified as AP-1 and AP-2 (16, 17).

In breast cancer cell lines phorbol esters inhibit cell proliferation (18, 19), but the effects of phorbol esters on estrogen receptor (ER) expression are unclear. It has been reported that TPA was either able (20) or unable (21) to influence the binding of 17-β-estradiol to intact MCF-7 cells. The principal aim of the present investigation was to study the effects of phorbol esters on ER expression and to determine the mechanism by which TPA regulates ER gene expression. To achieve this goal the relationship between ER protein concentration and binding capacity, the steady-state levels of receptor mRNA, and the level of ER gene transcription were examined simultaneously. The results presented herein demonstrate that treatment of MCF-7 cells with the phorbol ester TPA resulted in a decrease in ER protein and ER binding capacity. The decline in receptor protein to a new steady-state level accompanied a parallel decrease in the level of estrogen receptor mRNA. The metabolic inhibitor cycloheximide did not prevent the decrease in ER mRNA by TPA. The decrease in ER mRNA was not accompanied by a similar decrease in ER gene transcription, as determined by run-off experiments, suggesting that the predominant mechanism regulating ER expression is a post-transcriptional event. The half-life of the ER mRNA was determined, confirming that the effect of TPA was post-transcriptional. The decrease in ER levels induced by TPA is related to the activation of protein kinase C, as determined by the block of this effect by H-7, a known inhibitor of protein kinase C (22). Additionally, this report confirms the observation that TPA induces epidermal growth factor receptor (EGFr) and c-myc transcription.

MATERIALS AND METHODS

Tissue Culture—Monolayer cultures of MCF-7 breast cancer cells were grown in improved minimal essential medium supplemented...
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with 5% (v/v) charcoal-treated calf serum. The calf serum was pre-treated with sulfatase and dextran-coated charcoal to remove endogenous steroids. When the cells were 80% confluent the medium was replaced with phenol red-free improved minimal essential medium (23) containing 5% charcoal-treated calf serum. After 2 days in this condition, cells were re-treated and harvested at the time specified.

**Estrogen Receptor Assays** — For analysis of ER protein levels, MCF-7 cells were cultured and treated as described above. The level of receptor protein was assayed using an enzyme immunoassay kit from Abbott Laboratories (North Chicago, IL). To obtain total receptor protein the cells were homogenized by sonication in a buffer (50 mM Tris/HCl, pH 7.5, containing 5 mM EDTA, 10 mM EGTA, 0.3% (w/v) β-mercaptoethanol, 50 μg/ml phenylmethylsulfonyl fluoride, and 2 mM leupeptin). The homogenate was incubated on ice for 30 min and centrifuged at 100,000 × g for 1 h at 4 °C. Aliquots of the total extracts were then analyzed according to the manufacturer’s instructions.

To measure the level of estrogen binding sites, cells were incubated with [3H]estradiol under uncompeted and competed conditions as described previously (24). Specific binding activity was calculated as the difference between total and noncompetable [3H]estradiol binding. Binding constants and estrogen receptor concentrations were obtained by the method of Scatchard (25).

**Measurement of Protein Kinase C Activity** — For analysis of protein kinase C activity, MCF-7 cells were cultured and treated as described above. The level of protein kinase C activity was assayed using a specific peptide derived from the epidermal growth factor receptor (Amersham Corp.). To obtain total protein kinase C activity the cells were homogenized by sonication in a buffer (50 mM Tris/HCl, pH 7.5, containing 5 mM EDTA, 10 mM EGTA, 0.3% (w/v) β-mercaptoethanol, 50 μg/ml phenylmethylsulfonyl fluoride, and 2 mM leupeptin). The homogenate was incubated on ice for 30 min and centrifuged at 100,000 × g for 1 h at 4 °C. The supernatant was saved, and the pellet was treated for 20 min at 4 °C with the same buffer with the addition of 0.1% of Nonidet P-40 and centrifuged at 100,000 × g for 1 h at 4 °C. The second supernatant was combined with the first supernatant, and the protein kinase C activity was assayed using aliquots containing identical protein concentrations. The assay was performed according to the manufacturer’s instructions. To control for the specific incorporation of 32P into peptides, parallel samples were assayed in the absence of specific substrate, and the radioactivity was subtracted from specific samples. To eliminate 32P incorporated by kinases other than protein kinase C, parallel samples were assayed in the absence of Ca2+ and other activators of protein kinase C and for P, and for pS2 (31) have been described previously.

**Plasmids** — The clones for the estrogen receptor, pOR300 (26), Q7 (27), and pORS (27, 28), for the epidermal growth factor receptor, pE7150 (29), and from pE7150 using SP6 polymerase. Sixty micrograms of total RNA was hybridized for 12-16 h to the radiolabeled cRNA. After a 2-day treatment, cells were treated with TPA and 4-α-phorbol and incubated with [32P]UTP and unlabeled ATP, CTP, and GTP. The radiolabeled RNA transcripts were isolated and hybridized to an excess of denatured plasmid DNA immobilized on a nitrocellulose filter. The denatured plasmids used for the detection of specific transcripts were Q7, pOR3, 36B4, PBR322, c-myc exons 1 and 11, and pCHCegfr. Autoradiographs were analyzed by densitometry, and the background was subtracted. Results were normalized for the number of nuclei or by comparison with the transcriptional level of 36B4.

**RESULTS**

**Effects of TPA on ER Levels** — To determine the level of ER protein, an enzyme immunoassay (EIA) was employed. The data presented in Fig. 1 show that 100 nM TPA treatment resulted in a decline in total receptor protein by about 80%. Receptor protein declined from a level of approximately 235 fmol of ER/mg of protein in control cells to 50 fmol of ER/mg of protein in cells treated with TPA. The level of receptor decreased maximally by 24 h. The decrease in ER protein levels by TPA is a reversible response. After the removal of TPA, the normal level of ER protein was achieved by 48 h in fresh medium (data not shown). 4-α-Phorbol (100 nM), a compound related to TPA but lacking the biological effects of TPA, has no effect on ER protein (Fig. 1).

To confirm that the decreased level of ER protein, as measured by the EIA, corresponded to a decreased level of estrogen binding sites, a whole cell binding assay with increased amounts of [3H]estradiol (0.1-6 nM) was employed. The results of the EIA and competition assay are compared in Table I. In response to TPA treatment the number of estrogen binding sites decreased from 190 fmol of ER/mg of protein in control cells to 55 fmol of ER/mg of protein in 24-h treated cells. TPA alone had no effect on the binding assay. These results are similar to those reported previously for the effect of TPA on the level of estrogen binding sites (20). The level of binding decreased in a manner similar to the decline in 75 mM NaCl, 0.5 mM EDTA, 0.85 mM dithiothreitol, 0.125 mM phenylmethylsulfonyl fluoride, 50% glycerol. The concentration of nuclei was determined by diluting an aliquot in 0.4% (w/v) trypan blue and counting the number of nuclei. Nuclei were stored at −70 °C until the transcription elongation assay was performed.

**Transcription Elongation Assay and Isolation of RNA for Hybridization** — The nuclear transcription run-on assay was performed with a procedure described previously (32). Briefly, nuclei were isolated at various times after administration of TPA and 4-α-phorbol and incubated with [32P]UTP and unlabeled ATP, CTP, and GTP. The radiolabeled RNA transcripts were isolated and hybridized to an excess of denatured plasmid DNA immobilized on a nitrocellulose filter. The denatured plasmids used for the detection of specific transcripts were Q7, pOR3, 36B4, PBR322, c-myc exons 1 and 11, and pCHCegfr. Autoradiographs were analyzed by densitometry, and the background was subtracted. Results were normalized for the number of nuclei or by comparison with the transcriptional level of 36B4.

**FIG. 1. Effect of TPA and α-phorbol on the steady-state level of ER protein.** MCF-7 cells were grown in improved minimal essential medium supplemented with 5% charcoal-treated calf serum. At approximately 80% confluence, the medium was replaced with phenol red-free improved minimal essential medium containing 5% charcoal-treated calf serum. After 2 days, cells were treated with TPA (100 nM), α-phorbol (100 nM), or ethanol for various times. Cells were washed, harvested, and homogenized by sonication. Total estrogen receptor was determined using D547 and H222 monoclonal antibodies. Results are presented as fmol of ER/mg of protein. Each point is the mean of several experiments. ●, TPA; ○, α-phorbol.
Protein Kinase C Activity—To demonstrate that H-7 significantly blocks the TPA-induced decrease in ER protein, suggesting that protein kinase C plays a key role in the decrease in ER.

Effect of TPA Concentration on Estrogen Receptor and Protein Kinase C Activity—To determine the role of protein kinase C in the TPA-induced decrease in ER levels was investigated. To achieve this goal the effect of TPA on the level of ER protein was determined in MCF-7 cells after treatment with H-7 (10–40 μM), a protein kinase C inhibitor. The data presented in Fig. 2 show that H-7 by itself induces a slight increase in ER protein. The data also demonstrate that H-7 significantly blocks the TPA-induced decrease in ER protein, suggesting that protein kinase C plays a key role in the decrease in ER.

Effect of TPA Concentration on the Steady-state Level of ER mRNA—To obtain a maximum change in ER expression, the effect of 100 nM TPA and 100 nM 4-α-phorbol on the level of ER mRNA was measured using a RNase protection assay. In these experiments the level of receptor mRNA was normalized to the level of 36B4 mRNA, which is expressed constitutively in the presence of estradiol (29) and is not affected by TPA (data not shown). Changes in ER mRNA and 36B4 mRNA were quantified by scanning densitometry, and the data are pre-

| TABLE I
| Comparison of TPA effect on ER binding and protein |
| MCF-7 cells were grown as described in the legend to Fig. 1. Estrogen binding was determined by Scatchard analysis. ER was determined by the EIA. Mean ± S.E. (n). |
| Estrogen binding | ER |
| fmol/μg protein | fmol/μg protein |
| Control | 180 ± 9.81 (6) |
| 6 h | 90 ± 8.2 (4) |
| 24 h | 55 ± 6.5 (4) |

Fig. 2. Effect of TPA and H-7 on the steady-state level of ER protein. MCF-7 cells were grown as described in Fig. 1. After 2 days in phenol red-free medium containing 5% charcoal-treated calf serum, cells were treated with TPA (100 nM), H-7 (40 μM), or ethanol for various times. Total estrogen receptor was determined as described in Fig. 1. Results are presented as percent of control. Each point is the mean of several experiments. ■, TPA; □, H-7; ▲, TPA + H-7.

Fig. 3. Effect of TPA concentration on estrogen receptor and protein kinase C activity. MCF-7 cells were grown as described in Fig. 1. Panel A, protein kinase C activity was determined as described under “Materials and Methods” after treatment with different concentrations of TPA at 20 min, 3 h, and 24 h. The results are presented as pmol of 32P incorporated/min/unit of protein. Panel B, ER levels were determined by EIA as described in Fig. 1 after treatment with different concentrations of TPA for 3, 6, and 24 h. The results are presented as fmol of ER/mg of protein. Panel C, protein kinase C activity after treatment with 100 nM TPA for 20 min, 3 h, 6 h, and 24 h. Treatment and results are presented as described in Panel A. TPA concentrations: □, 5 nM; ◆, 10 nM; ■, 50 nM; and ○, 100 nM.

in receptor protein. No apparent change in the estrogen receptor affinity for estradiol was found in cells treated with TPA ($K_d = 1–3.5 \times 10^{-10}$ M).

To determine whether the effect of TPA was a universal effect or specific to MCF-7 cells, several ER-positive breast cancer cell lines were examined. In BT474 cells, TPA treatment resulted in a decline in ER protein whereas in T47D and ZR-75B cells, TPA treatment had no effect on ER expression. This result may be attributed to the low level of protein kinase C in the latter cells (33).

Effect of Protein Kinase C Inhibitors on ER Levels—The role of protein kinase C in the TPA-induced decrease in ER levels was investigated. To achieve this goal the effect of TPA on the level of ER protein was determined in MCF-7 cells after treatment with H-7 (10–40 μM), a protein kinase C inhibitor. The data presented in Fig. 2 show that H-7 by itself induces a slight increase in ER protein. The data also demonstrate that H-7 significantly blocks the TPA-induced decrease in ER protein, suggesting that protein kinase C plays a key role in the decrease in ER.

Effect of TPA Concentration on Estrogen Receptor and Protein Kinase C Activity—To determine the role of protein kinase C in mediating the effects of TPA, the concentration effect of TPA on protein kinase C activity and ER levels was measured. Concentrations of TPA from 5 to 100 nM activated protein kinase C in a dose-dependent manner (Fig. 3A). A more detailed time course shows that maximum activation of protein kinase C was achieved with 100 nM TPA by 6 h followed by a loss in activity by 24 h (Fig. 3C). Concentrations of TPA from 5 to 100 nM also resulted in the loss of ER in a dose-dependent manner (Fig. 3B). Maximum loss of ER was achieved with 100 nM TPA. After an initial lag period of about 3 h, the level of ER decreased up to 24 h. The time course of the TPA effects suggests that activation of protein kinase C, not its loss, is responsible for the decrease in ER (Fig. 3C).
presented as percent of control and are the mean of several experiments.

EGFr mRNA levels increased to approximately 2-fold. The values were obtained as the ratio of the integrated ER signal to the integrated 36B4 signal. The results are presented as percent of control. Panel A, time course of the effect of TPA and a-phorbol on the steady-state level of ER mRNA. Panel B, time course of the effect of TPA and a-phorbol on the steady-state level of EGFr receptor mRNA. TPA: O, a-phorbol.

**Fig. 4.** The effect of TPA on the steady-state level of ER mRNA. MCF-7 cells were grown as described in Fig. 1. After 2 days in phenol red-free medium containing 5% charcoal-treated calf serum, cells were treated with TPA (100 nM), a-phorbol (100 nM), or ethanol for various times. Total RNA was isolated, and 80 µg of total RNA was analyzed using a RNase protection assay. A 300-base pair fragment of the ER 32P-labeled antisense mRNA was protected against RNase A degradation by hybridization to total RNA. After hybridization at 30 °C for 12-16 h, total RNA was digested with RNase A. The protected bands were separated on 6% polyacrylamide gels; the bands were visualized by autoradiography and quantified by scanning densitometry. The values were expressed as the ratio of the integrated ER signal to the integrated 36B4 signal. The results are presented as percent of control. Panel A, time course of the effect of TPA and a-phorbol on the steady-state level of ER mRNA. Panel B, time course of the effect of TPA and a-phorbol on the steady-state level of EGFr receptor mRNA. TPA: O, a-phorbol.

**Fig. 5.** The effect of TPA and cycloheximide (CHX) on the steady-state level of ER mRNA. MCF-7 cells were treated as described in the legend of Fig. 1. Cells were grown in phenol red-free medium containing 5% charcoal-treated calf serum for 2 days and treated with TPA (100 nM) and/or cycloheximide (10 µg/ml). Total RNA was isolated and analyzed using a RNase protection assay as described in Fig. 4. The values were obtained as the ratio of the integrated ER signal to the integrated 36B4 signal. The results are presented as percent of control and are the mean of several experiments.

sented graphically in Fig. 4A as the ratio of integrated ER to the integrated 36B4 signal. In this study TPA treatment resulted in a maximum decrease in ER mRNA by 6 h. Treatment with TPA resulted in a decrease in ER mRNA levels to approximately 20% of control values; the level of ER mRNA remained low for up to 24 h. This is a reversible effect as removal of TPA results in the return of ER mRNA to control levels by 48 h (data not shown). The effect of TPA appears to be specific because 4-a-phorbol has no effect on ER mRNA (Fig. 4A). These data suggest that down-regulation of ER may be caused by a specific effect of TPA on ER mRNA. To determine that the effect of TPA on ER mRNA was not a result of a toxic effect on the cells, the effect of TPA on EGFr mRNA was measured as a positive control. It has been shown in other systems that TPA induces EGFr mRNA (34). In this study TPA treatment resulted in a maximum increase of EGFr mRNA by 24 h (Fig. 4B). After treatment with TPA, EGFr mRNA levels increased to approximately 14 times the control values, suggesting that the decline in ER mRNA was not caused by toxicity. The increase is not induced by 4-a-phorbol, suggesting that it is a specific effect of TPA (Fig. 4B).

The Effect of Cycloheximide and TPA on the Steady-state Level of ER mRNA—The effect of 10 µg/ml cycloheximide and 100 nM TPA on the steady-state level of ER mRNA was also examined using the RNase protection assay. Changes in ER mRNA were quantified by scanning densitometry, and the data are presented graphically in Fig. 5 as the ratio of the integrated ER to the integrated 36B4 signal. In this study cycloheximide alone had no significant effect on ER mRNA. The concentration of cycloheximide used inhibited protein synthesis by greater than 90% but had no effect on cell viability (data not shown). Treatment with TPA resulted in a maximum decrease in ER mRNA by 6 h. When assayed together, cycloheximide did not abolish the effect of TPA on ER mRNA, suggesting that the decline in ER mRNA is independent of protein synthesis.

**Effect of TPA on ER Gene Transcription**—To determine if the TPA-induced decrease ER mRNA was a transcriptional or post-transcriptional effect, nuclear transcription run-on assays were performed. The data presented in Fig. 6A show that 100 nM TPA does not affect the level of transcription of the ER gene. Although the data were normalized to 36B4 gene transcription, the same results were found when the values were normalized for the number of nuclei. As a positive control, the effect of TPA on EGFr and c-myc gene transcription was measured, and the data are shown in Fig. 6, B and C. TPA induces the transcription of EGFr (4-fold) and c-myc (approximately 4-fold) in MCF-7 cells. Taken together, these data suggest that the decrease in ER mRNA is caused by a post-transcriptional effect of TPA.

**Effect of TPA in ER mRNA Half-life**—To investigate the mechanism of post-transcriptional regulation, the half-life of the ER mRNA was determined. The data presented in Fig. 7 show that TPA decreased the half-life of the ER mRNA by a factor of 6 from approximately 4 h in control cells to 40 min in TPA-treated cells. These data suggest that the decrease in
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**FIG. 6. Effect of TPA and α-phorbol on ER gene transcription.** MCF-7 cells were treated as described in the legend of Fig. 1. Nuclei were isolated at the indicated times by homogenization in 1.5 M sucrose buffer containing 0.1% Brij 58; elongation of nascent transcripts was performed in a reaction buffer containing [35S]UTP. For detection of specific transcripts, the newly synthesized transcripts were isolated and hybridized to filters containing an excess of plasmid DNA. The level of transcription was determined by autoradiography and quantified by scanning densitometry. The level of transcription was expressed as the ratio of the integrated ER signal divided by the integrated 36B4 signal. The results are presented as percent of control. The values are the mean of several experiments. Panel A, effect of TPA and α-phorbol on ER gene transcription. Panel B, effect of TPA and α-phorbol on EGFr gene transcription. Panel C, effect of TPA and α-phorbol on c-myc gene transcription. ○, exon 1, TPA; □, exon 2, TPA; ▲, exon 1, α-phorbol; Δ, exon 2, α-phorbol.

**FIG. 7. Effect of TPA on ER mRNA half-life.** MCF-7 cells were treated as described in Fig. 1. After 24-h treatment with TPA (100 nM), transcription was stopped by the addition of 4 μM actinomycin D. Total RNA was isolated and analyzed by the RNase protection assay as described in Fig. 4. The ratio of the integrated ER mRNA signal to the integrated 36B4 mRNA signal was obtained. The values are presented as percent of control. Values are the mean of several experiments. ○, control; Δ, TPA.

ER mRNA is caused by a post-transcriptional effect of TPA on the stability of ER mRNA.

**DISCUSSION**

The purpose of the present study was to determine the mechanism by which phorbol esters regulate the expression of the estrogen receptor. To achieve this goal, the relationship between ER protein concentration and binding capacity, the steady-state levels of receptor mRNA, and the level of ER gene transcription were examined simultaneously. Previously, conflicting reports indicated that TPA was able (20) or unable (21) to decrease the level of ER protein and binding capacity. The results presented herein demonstrate that treatment of MCF-7 cells with TPA resulted in the decline in ER protein and binding capacity. The decline in receptor protein to a new steady-state level accompanied a parallel decrease in the level of receptor mRNA as reported recently by Lee et al. (34). The metabolic inhibitor cycloheximide did not prevent the TPA-induced decrease in ER mRNA. In contrast to the effect on ER protein and mRNA, TPA treatment had no effect on ER gene transcription. However, in the presence of TPA, the half-life of ER mRNA decreased by a factor of 6. These results suggest that the new steady-state level of receptor is largely determined by post-transcriptional regulation of ER mRNA. This regulation is independent of protein synthesis and appears to be mediated by the activation of protein kinase C. When cells are treated with increasing concentrations of TPA there is a corresponding increase in protein kinase C activity and a decrease in ER expression. The time course of the TPA effects suggest that protein kinase C activation, not its loss, is responsible for the decrease in ER expression.

We have reported previously that estradiol decreases the expression of ER in MCF-7 cells predominantly by a post-transcriptional mechanism (26, 35). Taken together these results clearly suggest that post-transcriptional regulation of ER mRNA plays a key role in determining the levels of ER in cells. It has become increasingly evident that post-transcriptional events are important for the maintenance of steady-state levels of many mRNAs. Post-transcriptional events such as processing of nuclear RNA, export from the nucleus, and mRNA degradation are recognized to be important in the regulation of cytoplasmic mRNA levels. The data presented here suggest that the post-transcriptional effects of TPA are to decrease the half-life of ER mRNA. Regulation of ER mRNA degradation appears to be mediated by protein kinase C independent of protein synthesis. Although the data suggest that ER mRNA degradation is independent of protein synthesis, the role of protein synthesis in mRNA degradation is not clear. Several mechanisms have been proposed to explain the role of protein synthesis in mRNA degradation. Inhibitors of protein synthesis have been shown to have varied effects on the stability of several mRNAs. Cycloheximide, an inhibitor of protein synthesis, has been shown to stabilize many unstable mRNAs, and the effect has been attributed to rapid loss of an unstable nuclease (36, 37). However, no direct evidence exists for such a protein. Studies using inhibitors of both peptide initiation and peptide elongation indicate that translation and polysome association play a role in mRNA degradation. Several genes require the progression of ribosomes through at least part of the coding sequence of the mRNA (38), suggesting that mRNA decay may be initiated by a ribosome-bound nuclease. Alternatively, the absence of translating ribosomes has been shown to result in the rapid decay of several mRNAs, suggesting an increased vulnerabil-
ity of the transcript not covered by ribosomes. Although the data presented here suggest that the effect of TPA on the post-transcriptional regulation of ER mRNA is mediated by protein kinase C independent of protein synthesis, the mechanism of ER mRNA decay awaits further investigation.

This report also confirms the induction of EGFr and c-myc expression in MCF-7 cells by TPA as reported previously in other cell types (10, 11). Interestingly, the 14-fold induction of EGFr mRNA by TPA is difficult to explain when the level of induction of EGFr gene transcription is only 4-fold. These effects appear to be independent of protein synthesis. Additionally, the ability of H-7 to block the effect of TPA suggests that the decrease in ER is caused by the activation of protein kinase C.

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