Auto-regulation of the Estrogen Receptor Promoter

C. G. Castles, S. Oesterreich, R. Hansen and S. A. W. Fuqua*

Department of Medicine/Division of Oncology, The University of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, TX 78284-7884, U.S.A.

The presence or absence of estrogen receptor (ER) plays a key role in the diagnosis and treatment of breast tumors. It is known that patients with breast tumors classified as ER-positive have a better prognosis. Observations such as this have led us to explore the question of what makes some breast tumors overexpress ER whereas others express either very low levels or none at all. To begin a study of ER regulation, we first chose to examine a 200 bp region of the ER promoter located immediately upstream from the transcribed sequence of the human ER gene. We found that this region of the ER promoter contained basal activity when transiently transfected into ER-negative HeLa cells. ER promoter activity was further increased by co-transfection of a wild-type ER expression vector, and this increased activity was hormone-dependent. Several ER deletion mutant constructs were also able to increase the activity of the ER promoter fragment, but none could support equivalent activity as was seen with the full-length ER. Therefore, we conclude that the ER can contribute to its own expression, and we hypothesize that this auto-regulation may contribute to its overexpression in some breast tumors. © 1997 Elsevier Science Ltd.

INTRODUCTION

The presence or absence of the estrogen receptor (ER) has long played a key role in the biology of breast cancer. The absence of ER in breast tumors is associated with a more aggressive disease course, whereas ER expression in tumors is associated with an improved prognosis and better therapeutic response to antiestrogens [1]. This has always been somewhat of a paradox, because it is known that very little ER is expressed in normal breast epithelium [2]. Observations such as these have led to much speculation as to why some tumors express the ER, whereas others do not. Some of the earliest speculation centered around whether the simple loss of the ER gene was responsible for the ER-negative phenotype; but complete loss of the gene has not been reported in any breast cancer specimens to date [3,4]. It thus appears that transcriptional regulation may be responsible for the lack of expression in ER-negative tumors. There does remain, however, some disagreement as to whether gene amplification is present in breast tumors [5,6]. Much of the speculation about ER expression in breast cancer has turned to the analysis of the upstream promoter region of the ER gene, of which over 3000 bases have now been sequenced [7,8]. These studies have revealed the presence of an additional upstream exon (called exon 1') having open reading frames from which at least two mRNA isoforms (ER mRNAs 2 and 3) [7,8] arise, in addition to the one (ER mRNA 1) which is the predominant transcript in most ER-containing tissues [9,10].

Two binding sites for a novel DNA-binding protein found in ER-positive breast and endometrial cancers, but not ER-negative breast cancers, were identified in a 75 bp region of the 5' untranslated leader of the ER transcript [11]. This leader sequence also contains a CpG island which was found to be hypermethylated in ER-negative, but not ER-positive breast cancer cells [12]. It has also been demonstrated that the ER promoter region between the cap site at +1 of the ER mRNA 1 [9] and exon 1' contains a stretch of 1884 bp with numerous putative regulatory elements. It thus appears that both the 5' untranslated leader and the 5' upstream promoter region of the ER gene may play key roles in the expression of the ER in different tumors. Therefore, as is the case with
many other genes, ER regulation may be controlled or influenced by a number of different cis-acting control elements scattered throughout the gene. Although it is known that the ER, usually upon activation by estrogens, regulates the expression of certain genes such as the progesterone receptor, TGF-α, and pS2, it is not yet known what role the ER may play in its own regulation. It is generally accepted that estrogen can down-regulate ER expression [13] in breast cancer cells, and others have shown that estrogen can also up-regulate ER expression in some cells [14], but little is known about the mechanism(s) involved in these events. One potential mechanism is that the ER may be involved in regulating its own expression, and this regulation may be complex due to the diverse transcriptional activities exhibited by different domains of the receptor. Another potential level of complexity is the expression of certain variant forms of the ER which contain alterations within different ER functional domains [15].

Among these ER domains is a hormone-dependent transactivating function known as AF-2 located within the hormone-binding domain (HBD) of the ER, and a hormone-independent transactivating function (AF-1) found in the NH2-terminal region of the receptor [16–18]. It is now apparent that these two major transactivating activities of the ER can differ depending on the cellular and/or promoter context of the system [19]. For example, sometimes both these domains are necessary to activate a given promoter, whereas in other cells, these domains can function independently. Another hormone-independent activation function called AF-2a has been identified within the HBD of the ER [20], bestowing further complexity to the transcriptional activities of the ER.

In order to examine the ability of estrogen and/or the ER to influence the activity of its own expression, we first had to determine which region of the upstream promoter region was active in transcriptional activation of heterologous promoters. We began by examining a 231 bp fragment of the ER promoter just upstream from the +1 cap site. Transient transfection of this fragment into ER-negative HeLa cells resulted in a low level of promoter activity. However, co-transfection of wild-type ER (WT ER) into these cells dramatically increased the activity of the ER promoter. We used a panel of ER deletion expression vectors to delineate the regions of the receptor that are critical to the ER-mediated increase in promoter activity. We found that the AF-2 domain was most important for this up-regulation, and that this activity was not dependent on a direct DNA:protein interaction of the ER with this region of its promoter.

**MATERIALS AND METHODS**

**Expression plasmid constructions**

Oligonucleotide primers (nucleotides −9 to −28 and −222 to −239) based on previously published sequence information for the upstream region of the ER [7–9] were used to generate ER promoter fragments from normal placental DNA by polymerase chain reaction (PCR) using a method previously described [21]. A 231 bp ER promoter expression vector (ERP-1) was created by cloning this PCR-generated product into the XhoI-KpnI sites of the promoterless luciferase reporter plasmid pGL-2 basic (Promega, Madison, WI, U.S.A.). A full-length WT ER expression vector was also constructed by subcloning the BamHI–EcoRI fragment from the yeast expression vector YEPE10 [22] into the pcDNAI expression vector (Invitrogen, San Diego, CA, U.S.A.). A fragment containing the DNA-binding domain (DBD)
Auto-regulation of the Estrogen Receptor Promoter

and AF-2 domains of the ER (ER DBD/AF-2) was generated by PCR using sense (nucleotides 671–690 of the ER) and antisense (nucleotides 2024–2008) [9] ER-specific primers, and then cloned into the EcoRI–SphI sites of the pcDNA1 expression plasmid. Expression constructs for the three ER/Gal4 fusions were kindly provided by Dr. Z. Nawaz (Baylor College of Medicine, Houston, TX, U.S.A.). These fusion vectors were made by inserting specific domains of the ER as indicated, which had been PCR-amplified from the yeast ER expression vector YEPE10 [22] into the pABgal94 plasmid [40] which contains amino acids 1–94 of the Gal4 DBD.

Cell culture and transfections

HeLa cells were maintained in MEM complete media supplemented with 10% fetal bovine serum (FBS; Sigma, St Louis, MO, U.S.A.). Cells were plated into six-well cluster dishes (Falcon, Franklin Lakes, NJ, U.S.A.) at a density of 8 x 10^4 cells/well and allowed to recover for 24 h. Transient transfections were then carried out in a total of 1 ml OptiMEM reduced-serum media using 8 µl of lipofectamine/well as per manufacturer’s instructions (Gibco/BRL, Gaithersburg, MD, U.S.A.). Transfections of individual wells were performed using 1 µg of luciferase reporter plasmid (ERP-1 or pGL-2 basic vector alone), 100 ng of a CMV-driven β-galactosidase (β-gal) plasmid (a kind gift of Dr. Michael McPhaul) as a correction for transfection efficiency, and 25 ng of either an ER-containing expression plasmid or the pcDNA1 vector alone control. Following a 6 h incubation with the DNAs, the transfection media was removed and 2 ml of phenol red-free MEM supplemented with 10% charcoal-stripped FBS was added. After an additional overnight recovery, the cells were treated with either 10^-8 M estradiol or ethanol vehicle for 22 h. Cells were then harvested, the luciferase assays were performed using a commercial kit (Promega), and the β-gal assays were performed using the method of Rouet et al. [23]. Results are shown as fold activity over control activity of the promoterless pGL-2 basic vector in each set of experiments. All transfections and assays were performed in duplicate with n>3 individual experiments.

Gel-retardation assay

Nuclear extracts [24] were prepared from HeLa cells for gel-retardation analysis. As a positive control, non-radioactive in vitro translated ER was prepared using the TNT R Coupled Reticulocyte Lysate System (Promega) as per manufacturer’s instructions. DNA-binding reactions were carried out in buffer containing a 32P-labelled 231 bp DNA probe consisting of the entire ERP-1 promoter fragment, 3 µg poly(dA•dT), 1.2 mM DTT, 12 mM Tris HCl pH 7.5, 12% glycerol, 100 mM KCl and 10 µg nuclear extract or 2 µl IVT-ER in a 20 µl total volume at room temperature for 30 min [25]. One microgram of the ER311 mAb (NeoMarkers, Inc., Fremont, CA, U.S.A.) which recognizes the HBD of the ER was added and the samples were then incubated for 2 h at 4°C before being separated at 100 V on a 4% polyacrylamide gel maintained at 4°C.

RESULTS

The ERP-1 promoter fragment possesses low basal activity

The ERP-1 promoter fragment was cloned into the promoterless pGL2-basic vector upstream of a luciferase reporter gene and transiently transfected into the ER-negative HeLa cell line. HeLa cells were chosen because of their high transfection efficiency. Figure 1 illustrates the structure of the ERP-1 region (−9 to −239 bp) and potential promoter sites which contain, including a CAT box, an Sp1 site, a TFIID binding site, and one-half of a consensus estrogen response element (1/2 ERE). This region is far downstream from

![Fig. 2. The upstream region of the ER exhibits promoter activity which is increased by hormone-activated receptor. An expression vector containing ERP-1 as described in Fig. 1 was transfected into HeLa cells, which are devoid of ER expression, either alone (ERP-1) or co-transfected with WT ER (ERP-1+ WT ER) or control pcDNA vector (ERP-1+ pcDNA control). Luciferase assays were performed as described in Materials and methods. Values are presented as fold over control based on the luciferase activity of the pGL-2 basic vector alone control after correcting for transfection using β-gal.](attachment:image)
the other reported open reading frames [7, 8] extending upstream starting at -1884 (exon 1').

As seen in Fig. 2, the ERP-1 promoter fragment exhibits basal activity that is approximately two-fold over that of the pGL2-basic vector alone control, and as might be expected, this activity was not influenced by the addition of exogenous estradiol. There thus appears to be very low basal promoter hormone-independent activity within this most proximal region of the ER gene. When the ERP-1 construct was co-transfected with a WT ER expression vector, the promoter activity of ERP-1 increased 10-fold over that seen with the vector alone control (Fig. 2, ERP-1+ WT ER) and this increase was dependent on the addition of estradiol. Co-transfection of the expression vector (pcDNAI) alone lacking the WT ER sequence failed to increase ERP-1 activity. The slight increase in ERP-1 promoter activity in cells expressing WT ER but lacking hormone may be attributable to the very low levels of endogenous hormone still remaining in the culture media. Similar results were obtained with the ER-positive breast cancer cell lines MCF-7 and T47D; a two-fold increase in activity was seen with ERP-1 over that with the vector alone when estradiol was added, and this activity could be increased to three- to six-fold if exogenous WT ER

was also co-transfected into these cells (results not shown). However, because of the low transfection efficiencies of these two cell lines compared with HeLa cells, we elected to continue only with the HeLa cell line.

We conclude that since hormone-activated WT ER was required for the promoter activity of ERP-1, it is quite possible that at least in some cellular contexts, ER may auto-regulate its own expression. However, this auto-regulation may involve other segments of the ER promoter, as evidenced by our finding that a 2.7 kb ER promoter fragment inclusive of much of the exon 1' shown in Fig. 1 exhibited approximately two- to three-fold increased activity as compared to the promoter activity seen with the ERP-1 region in the presence of WT ER and estradiol (data not shown). However, we have elected here to focus only on the smallest region of the ER promoter (ERP-1) which is influenced by ER co-expression first to define the basal promoter region before expanding to larger regions of the promoter.

A region within AF-2 is required for ER promoter activity

In order to begin characterizing this potential autoregulation, we next utilized a panel of ER variants, both deletion mutants generated in vitro and the natu-
rally occurring exon 5 ER deletion variant [22] to delineate the regions of the receptor critical to this increase in ERP-1 promoter activity. Figure 3 diagrams the panel of ER mutants used in our transfection experiments, along with the relative positions of the AF-1, DNA-binding, and AF-2 domains of the receptor. To determine if the AF-2 region of the ER was sufficient for activation of the ERP-1 promoter fragment, a cDNA containing only the DBD and the HBD of the ER was generated (ER DBD/AF2). This construct increased the promoter activity of ERP-1 four- to five-fold over that seen with the vector-alone control (Fig. 4, ERP-1+ ER DBD/AF-2) when it was co-transfected into HeLa cells. As expected, this increase was hormone-dependent. However, the overall promoter activity of the ER DBD/AF-2 mutant was only about one-half of that obtained with expression of full-length WT ER (Fig. 2).

To determine whether the DBD of the ER was required for promoter activation, we next used a construct in which the DBD of Gal4 was substituted for the ER DBD (this plasmid was called Gal4 DBD/ER AF-2 and lacks the AF-1 domain of the ER) (see Fig. 3). As a negative control, an expression vector containing only the Gal4 DBD was also used. Co-expression of Gal4 DBD/ER AF-2 was able to increase ERP-1 activity in a hormone-dependent manner (Fig. 4), but at a level which was slightly below that of the ER DBD/AF-2 mutant. This suggested that the ER DBD was not an absolute requirement for activation of the ERP-1 promoter fragment. This conclusion was further illustrated in that a construct

Fig. 4. The activity of the ERP-1 region of the ER promoter can be increased independently by different functional domains of the ER. ER mutant expression constructs were co-transfected with the ERP-1 luciferase expression construct into HeLa cells, and pGL-2 basic vector alone (vector control) was used to normalize the promoter activity of ERP-1. ER mutants included one which contained only the DBD and AF-2 domains (□), a similar mutant in which the ER DBD was replaced by the DBD of Gal4 (▲), and a control for Gal4 DBD activity which contained only this region (■). Other mutants included one having the AF-1 domain of the ER but the Gal4 DBD (△), and another containing both the AF-1 and AF-2 ER activation functions but having the Gal4 DBD (□). The promoter activity of the naturally occurring exon 5 ER deletion variant (○) was also examined. Separate sets of experiments were performed for each ER mutant with each set having its own pGL-2 basic and pcDNAI vector alone controls, as well as an ERP-1 alone control. Fold over control values were calculated for ERP-1 alone and each ER mutant based on normalization to its own internal pGL-2 basic control samples. As was the case with the WT ER (Fig. 2), for each mutant, all values for pcDNAI control samples were equal to that of the ERP-1 alone (not shown).
containing both the AF-1 and AF-2 domains, but substituted with a Gal4 DBD (ER AF-1/Gal4 DBD/AF-2), was also able to activate the ERP-1 promoter fragment (Fig. 4). Interestingly, this receptor chimera exhibited activity which was both estrogen-independent (three-fold over the vector alone control) and estrogen-dependent (six-fold), suggesting that the ER DBD does indeed play a role in imparting full promoter activity to the WT ER.

The ability of the hormone-independent AF-1 domain to activate the ERP-1 promoter fragment was further assessed with another Gal4 chimera, termed ER AF-1/Gal4 DBD (diagrammed in Fig. 3). This construct was devoid of activity (Fig. 4), suggesting that the AF-2 domain is absolutely required for ERP-1 promoter activity. A titration experiment using the ER AF-1/Gal4 DBD expression vector from 10–50 ng also failed to influence ERP-1 promoter activity (data not shown).

**Ability of a naturally occurring ER variant to activate ERP-1**

Previous studies by our group have identified and characterized a variant ER mRNA identified from human breast tumors in which AF-1 is left intact, but exon 5 is precisely deleted, leading to the expression of a truncated receptor lacking most of the hormone binding and AF-2 domains [22, 26] (diagrammed in Fig. 3). This variant ER exhibits constitutive activation of consensus estrogen response elements in yeast cells [20]. We found that co-transfection of the exon 5 ER deletion variant with the ERP-1 fragment increased the promoter activity of ERP-1 four- to five-fold over that seen with the vector-alone control (Fig. 4). However, in contrast to the activity induced by AF-2 (ER DBD/AF-2, Fig. 4), the increase in the activity seen with the exon 5 ER deletion variant construct was estrogen-independent. Therefore although AF-2 alone can increase ERP-1 promoter activity, AF-1 can only activate ERP-1 in the context in which the ER DBD and/or the proximal portion of the HBD domain are also present. Further studies should allow greater precision of the exact location of these potential key sequences in ERP-1 activation.

**Binding analysis using ERP-1 DNA**

There is clearly promoter activity contained within the ERP-1 fragment which is influenced by ER co-expression, and we therefore began to search for potential binding proteins that might interact with this fragment, including the hypothesis that the ER itself may directly bind this region. Gel-retardation analysis was performed on nuclear extracts (NE) prepared from HeLa cells and on in vitro translated WT ER (IVT-ER) using the entire 231 bp of the ERP-1 promoter region as a probe (Fig. 5). The band in the first lane of Fig. 5 (shown by arrow A) identifies a protein, or protein complex, which bind(s) to the ERP-1 region. This band failed to be up-shifted by an ER-specific antibody (second lane), suggesting that this protein–DNA complex does not contain ER. This was further substantiated by attempts at directly interacting IVT-prepared ER with the ERP-1 fragment; the uncomplexed ERP-1 probe is seen at the bottom of each of these three lanes. As a positive control for the IVT-ER, analyses were performed with the consensus ERE probe, and identified a band (arrow B) which was up-shifted (arrow C) upon the addition of ER-specific mAb and eliminated following the addition of cold competitor ERE.

![Fig. 5. Gel retardation analyses of HeLa cell nuclear extracts (NE) or in vitro-translated ER (IVT-ER). Analyses were performed with nuclear extracts of HeLa cells and the entire ERP-1 promoter region. The arrow A identifies a band representing a protein (or proteins) which binds to the ERP-1 region but is not upshifted by an ER-specific mAb. Analyses of IVT-ER using the ERP-1 fragment revealed no band, and the uncomplexed ERP-1 probe is seen at the bottom of each of these three lanes. As a positive control for the IVT-ER, analyses were performed with the consensus ERE probe, and identified a band (arrow B) which was upshifted (arrow C) upon the addition of ER-specific mAb and eliminated following the addition of cold competitor ERE.](image-url)
transcription factor(s) present (arrow A) which may then influence ER promoter activity.

**DISCUSSION**

In considering the question of ER regulation in breast cancer, there are two important facts which must be considered. First, the majority of breast tumors express higher levels of ER compared to adjacent normal breast epithelium [27,28]. Second, 30–40% of breast tumors fail to express ER at the protein or mRNA level [29,30]. In general, patients with ER-positive tumors have a longer disease-free interval and better overall survival compared to patients whose tumors are classified as ER-negative [31,32]. Even so, the question still remains: What is inducing high levels of ER expression in some breast tumors?

Although several different mechanisms, post-transcriptional and translational, are probably involved in controlling ER expression in breast carcinomas, it appears that transcriptional regulation may be the major factor responsible for the lack of expression in ER-negative tumors. Weigel et al. [33] were unable to detect ER mRNA in ER-negative tumors using the very sensitive techniques of Northern hybridization and reverse transcriptase-PCR, and they were able to demonstrate that the ER gene was not lost using Southern hybridization analyses. In addition, it was recently shown that methylation of a cytosine-rich CpG island within the leader and first exon of the ER gene was associated with a failure to express ER in human breast cancer cell lines [12]. Recently, direct demethylation experiments targeting the ER promoter using the DNA methylation inhibitors, 5-azacytidine and 5-aza-2'-deoxycytidine, led to the re-expression of ER mRNA, and the restoration of ER protein synthesis in the ER-negative MDA-MB-231 human breast cancer cell line [34]. This suggests that methylation of this region may play an important role in the transcriptional regulation of ER in ER-negative breast cancer cells.

Evidence for transcriptional regulation of the ER in ER-positive breast cancer has recently been described by deConinck et al. [11]. In this study, they identified a region within the 5' ER untranslated leader which contributed to promoter activity in breast cancer cell lines. This region contains sites within the leader for a potential DNA-binding protein which was expressed only in ER-positive breast cancer cell lines. This DNA-binding protein may also play a role in ER transcriptional regulation, again underscoring the complexity of ER regulation. Therefore, as is the case with many other genes, ER regulation may be controlled or influenced by a number of different cis-acting control elements scattered through the putative 5' region.

It is well established that transcription by RNA polymerase II requires cis-acting regulatory elements for basal activity in mammalian cells. The most common proximal element found in promoters recognized by RNA polymerase II is the TATA sequence located upstream from the cap site [35]. The TATA sequence is then followed by one or several elements positioned within the first 200 bp. The studies described above [11] focused on the region of the ER 5' untranslated leader which is downstream from the cap site of the ER gene. In contrast, we have focused on an area of the promoter just upstream of the cap site, beginning at -9 and extending to -239 bp which is commonly the region of important cis-acting elements.

We show that the WT ER may play a role in up-regulating its own transcription at the level of the promoter, and that this regulation appears to be dependent on the binding of hormone. Previous studies have shown that ligand-activated ER can also transiently down-regulate its own mRNA, presumably by inhibition of ER gene transcription or post-transcriptional mechanisms [13,36]. However, because these studies utilized the highly ER-positive MCF-7 breast cancer cell line, and these effects were rapid and transient in nature, these findings may not conflict with those we described in ER-negative HeLa cells.

Numerous studies over the past few years have focused on the role of naturally occurring ER variants in breast cancer progression. Some ER variants fail to bind DNA, and a few have been shown to inhibit WT ER transcriptional activity in a dominant manner [37,38]. The exon 5 ER deletion variant is able to activate gene transcription constitutively, and we have postulated that it may play a role in the development of hormone resistance [22,26]. Some of the described variants lack large portions of the receptor, such as the HBD [22], whereas others may lack the DBD or portions of the AF-1 region [25,39]. We hypothesized that if hormone-activated WT ER could enhance the promoter activity of ERP-1, then ER variants might have important differential effects on ER auto-regulation. Our data indicates that the AF-1 region of the ER alone fails to influence ERP-1 promoter activity, whereas the AF-2 domain in the carboxyterminus of the ER increases ERP-1 activity in a hormone-dependent manner. The ability of AF-2 to influence promoter activity does not appear to be dependent on the presence of the ER DBD, although the DBD appears to be required for full activity of the wild-type receptor on ERP-1. The failure of the AF-1 domain alone to increase ERP-1 activity may signify a dependence on the presence of an intact DBD, or most probably the proximal portion of the ER HBD. This is suggested by the hormone-independent increase in ERP-1 activity by the naturally occurring exon 5 ER deletion variant, which is lacking the majority of the AF-2 domain, but which retains this proximal portion of the HBD.

It is of interest to note that the exon 5 ER deletion variant increased the promoter activity of ERP-1 at a
level equivalent to that seen with the ligand-activated ER DBD/AF-2 mutant. However, both of these deletion mutants exhibited less activity than the full-length ER. Because maximum ER promoter activity was dependent on the expression of the WT ER, it is possible that taken together, the exon 5 deletion variant and the ER DBD/AF-2 mutant could each account for a portion of the activity of the wild type, as their individual activities added together would total about 10-fold over the vector control. It is also possible that an overlapping region contained by both mutants could be responsible for most of the promoter activity obtained experimentally. For example, a region of the ER which is purported to possess a hormone-independent constitutive activation function (AF-2a) is contained within both the exon 5 deletion variant and DBD/AF-2 ER mutants [20]. However, initial transient assays involving a construct containing only the ER DBD and AF-2a regions have failed to demonstrate any increase in ERP-1 activity (data not shown). Although we cannot yet conclude that ER auto-regulation is dependent on any particular domain of the receptor, it is apparent that maximal activation of the ERP-1 promoter region by WT ER is dependent on the conformation of an intact receptor that includes all of the major domains.

Gel-retardation analysis using the ERP-1 region as a probe identified an unknown protein/DNA-binding complex which failed to upshift with an ER antibody, yet was competed effectively with excess cold consensus ERE oligonucleotides. In contrast, WT ER protein was unable to bind the ERP-1 region, indicating that WT ER does not directly bind to this region of the ER promoter. The presence of a half-ERE site along with transcriptional motifs within the ERP-1 such as a TFIID binding site, an Sp1 site, and a CAT box sequence, suggests that protein–protein interactions may be important mechanisms underlying ER auto-regulation of ERP-1. Indeed, it is becoming increasingly apparent that hormonal regulation of some estrogen-responsive genes does not require direct DNA binding or the DBD of the ER, supporting the hypothesis that protein interactions might be involved [41, 42].

In conclusion, the ERP-1 region of the ER promoter exhibits low basal activity in HeLa cells which is enhanced upon the addition of ligand-activated WT ER. This induction is dependent on the expression of most or all of the wild-type ER, possibly due to conformational requirements involving specific receptor domains. Whereas AF-2 alone can increase ERP-1 activation following estradiol treatment, AF-1 can only activate ERP-1 in the context in which the ER DBD and/or the proximal AF-2a portion of the HBD are also present, and this activity is hormone-independent. The mechanism of this ER auto-regulation may be as yet unknown protein–protein interactions between the receptor and other transcription factors which bind to its promoter. Further studies will be needed to link this potential mechanism of auto-regulation directly with the regulation of ER in breast cancer.

Acknowledgements—Supported by NIH grants CA30195 and P30 CA54174. The authors would especially like to thank Zafar Nawaz for the Gal4/ER constructs.

REFERENCES
