Cell- and Ligand-specific Regulation of Promoters Containing Activator Protein-1 and Sp1 Sites by Estrogen Receptors α and β*

Jennifer R. Schultz‡*, Larry N. Petz§, and Ann M. Nardulli†
From the Department of Molecular and Integrative Physiology, University of Illinois, Urbana, Illinois 61801

Estrogen plays a critical role in development and maintenance of female reproductive and mammary tissues, but is also involved in maintenance of cardiovascular, skeletal, and neural function. Although it is widely accepted that the estrogen-occupied receptor mediates its effects by interacting with estrogen response elements (EREs) residing in target genes, a number of estrogen-responsive genes contain no identifiable ERE. To understand how estrogen-responsive genes lacking EREs but containing activator protein 1 (AP-1) and Sp1 sites respond to hormone treatment, we have identified four discrete regions of the human progesterone receptor gene that contain AP-1 or Sp1 sites and examined their abilities to modulate transcription in the presence of 17β-estradiol, ICI 182,780, tamoxifen, raloxifene, genistein, or daidzein. Transient cotransfection assays demonstrated that ERα was a more potent activator of transcription than ERβ in bone, uterine, and mammary cells. The Sp1-containing promoters were substantially more potent transcriptional enhancers than the AP-1-containing promoters, but a 1.5-kb region of the human progesterone receptor gene containing both AP-1 and Sp1 sites was the most hormone-responsive promoter tested. The ability of ligands to modulate transcription of AP-1- or Sp1-containing promoters was dependent on cell context, but the expression of AP-1 or Sp1 proteins was not necessarily related to transcriptional response. Taken together, these studies have helped to delineate the roles of ERα and ERβ in modulating transcription of genes containing AP-1 and Sp1 sites and define the effects of widely used, pharmacologic agents in target cells with distinct cellular environments.

Estrogenic hormones are required for growth and differentiation of female reproductive tissues, contribute to male fertility, and play a role in maintaining cardiovascular, skeletal, and neural cell function (1–8). Estrogenic and antiestrogenic hormones have been widely used to regulate fertility, relieve postmenopausal symptoms, and decrease the incidence and recurrence of mammary tumors. Because the selective estrogen receptor modulators tamoxifen and raloxifene have agonistic effects in bone and antagonistic effects in mammary cells, they have been used to decrease breast cancer incidence and recurrence (9, 10) and maintain bone mineral density (11, 12). However, the agonistic effects of tamoxifen in the uterus have been associated with an increased incidence of endometrial cancer. ICI 182,780 has been classified as a pure antagonist and has been used successfully to treat women with tamoxifen-resistant breast cancer (13). The soy phytoestrogens genistein and daidzein are used to help alleviate postmenopausal symptoms. Consumption of these phytoestrogens has been linked to a reduced risk of breast cancer (14). In addition, genistein, which binds preferentially to estrogen receptor (ER)β and influences mammary tumor formation in rodents, also improves plasma lipid profiles in humans (15–17). The widespread use of estrogens, selective estrogen receptor modulators, and phytoestrogens as pharmacologic agents to treat women with postmenopausal symptoms, to prevent breast cancer, and to limit breast cancer recurrence (18–20) provides compelling impetus to define how these hormones regulate gene expression.

The effects of estrogens are mediated by the two ERs, ERα and ERβ, which are expressed in reproductive as well as nonreproductive tissues (21–23). These two receptors bind to a variety of ligands and display tissue-specific effects. Whereas classical models of estrogen action have proposed that ERα and ERβ influence gene expression by binding to ligand and then to estrogen response elements (EREs) in DNA (21, 24), many estrogen-responsive genes do not contain classical EREs. Recent studies have shown that ERα can influence gene expression by interacting with AP-1 and Sp1 proteins (25, 26). ERα interacts directly with Jun (27, 28) and enhances binding of AP-1 proteins to their recognition sites in DNA (29, 30). Furthermore, AP-1-mediated transcription of the ovalbumin, c-fos, collagenase, and interleukin growth factor 1 genes is enhanced in an estrogen- and ERα-dependent manner (31–34). A number of estrogen-responsive genes contain Sp1 sites adjacent to an imperfect ERE or to an ERE half-site such as the c-myc and retinoic acid receptor α genes (35, 36). At these sites, ERα is able to bind directly to DNA and enhance Sp1 binding to its recognition site. Other estrogen-responsive genes including the urooglobin, cathepsin D, bcl-2, fos, and adenosine deaminase genes (26, 37–40) contain Sp1 sites, but lack any ERE half-sites. Thus, estrogen responsiveness can be mediated by the interaction of ERs with DNA or with DNA-bound transcription factors.

The human progesterone receptor (PR) gene is regulated by

Received for publication, July 13, 2004, and in revised form, October 22, 2004
Published, JBC Papers in Press, October 26, 2004, DOI 10.1074/jbc.M407879200

* This work was supported in part by National Institutes of Health Grants DK 53844 and DK 61469 (to A. M. N). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
‡ Supported by predoctoral National Institutes of Health Cell and Molecular Biology Training Program Grant T32 GM07283, National Institutes of Health Reproductive Biology Training Program Grant PHS ST32 HD07028, and a Susan G. Komen Breast Cancer Foundation Dissertation Fellowship.
§ Supported by postdoctoral National Institutes of Health NIEHS Training Program Grant ES07326.
† To whom correspondence should be addressed: Dept. of Molecular and Integrative Physiology, University of Illinois at Urbana-Champaign, 524 Burrill Hall, 407 South Goodwin Ave., Urbana, IL 61801. Tel.: 217-244-5679; Fax: 217-333-1133; E-mail: anardulli@life.uiuc.edu.

The abbreviations used are: ER, estrogen receptor; AP-1, activating protein-1; ERE, estrogen response element; E2, 17β-estradiol; PR, progesterone receptor; CAT, chloramphenicol acetyltransferase; HEC, human endometrial carcinoma; ANOVA, analysis of variance.
estrogen (41–43) and, like a number of other estrogen-responsive genes, does not contain a palindromic ERE. We have, however, identified four distinct regions in the human PR gene that confer estrogen responsiveness in a ligand- and ERα-dependent manner. Two of these regions, the −61 Sp1 site and the +571 ERE/Sp1 site, contain tandem Sp1 sites alone or adjacent to an ERE half-site, respectively (44, 45). ERα enhances Sp1 binding to these sites and is required for estrogen responsiveness. ERα also binds directly to the ERE half-site in the +571 ERE/Sp1 site to augment the effect of estrogen. The other two regions, the +90 AP-1 site and +745 AP-1 site, bind to Fos and Jun and ERα enhances binding of these AP-1 proteins to these two sites (29, 30). To better understand how genes containing AP-1 and Sp1 sites are regulated by estrogenic and antiestrogenic ligands, we have compared the abilities of ERα and ERβ to activate transcription of reporter plasmids containing an AP-1 or Sp1 site alone or in combination in three different cell contexts.

**EXPERIMENTAL PROCEDURES**

*Cell Maintenance and Transient Transfections—*Human osteosarcoma (U2-OS) cells were maintained in culture and transfected using Lipofectin (Invitrogen, Carlsbad, CA) as previously described (46). Human endometrial carcinoma cells (HEC-1) cells were maintained in Eagle’s minimum essential medium containing 5% heat-inactivated calf serum and 5% heat-inactivated fetal calf serum. Cells were plated in phenol red-free improved minimal essential medium supplemented with 5% charcoal/dextran-treated fetal calf serum 24 h prior to transfection and were transfected in serum-free, phenol red-free improved minimal essential medium. After transfection, the DNA/media mixture was removed and cells were maintained on phenol red-free improved minimal essential medium supplemented with 5% charcoal dextran-treated calf serum. Human breast cancer cells (SK-BR-3) were maintained in Dulbecco’s modified Eagle’s medium/F-12 supplemented with 10% heat-inactivated fetal calf serum. Cells were plated in Dulbecco’s modified Eagle’s medium/F-12 supplemented with 10% charcoal/dextran-treated fetal calf serum 24 h prior to transfection and were transfected in serum-free Dulbecco’s modified Eagle’s medium/F-12. After transfection, the DNA/media mixture was removed and cells were maintained on Dulbecco’s modified Eagle’s medium/F-12 supplemented with 10% charcoal/dextran-treated fetal calf serum. For transfections utilizing Sp1 expression vectors, SK-BR-3 cells were maintained in McCoy’s 5A medium supplemented with 10% heat-inactivated fetal calf serum. Cells were plated in McCoy’s 5A supplemented with 5% charcoal/dextran-treated calf serum. Cells were transfected with 25 or 100 ng of the CAT reporter vector with or without 10 or 100 ng of an Sp1 expression vector (kindly provided by Tom Korgopp, University of Michigan, Ann Arbor, MI, Ref. 49). The reporter plasmids each included a TATA box (TATA-CAT, Ref. 50) alone, with 1.5 kb of the PR gene (−711/+817 PR TATA-CAT, Ref. 30) or with a region of the PR gene containing two adjacent Sp1 sites (−61 Sp1 TATA-CAT, previously referred to as −80/−34 TATA-CAT, Ref. 45), two tandem Sp1 sites and an adjacent ERE half-site (−571 ERα/Sp1 TATA-CAT, Ref. 44), or an AP-1 site (+90 AP-1 TATA-CAT) (29) and +745 AP-1 TATA-CAT (30). Cons ERβ +10 TATA-CAT, which contained a consensus ERE from the Xenopus laevis vitellogenin A2 gene 36 bp upstream of a TATA box was also utilized for comparison (51). Cells were transfected for 8 h, after which they were treated with ethanol vehicle, 10 nm 17β-estradiol (E2), or 100 nm 4-hydroxytamoxifen (TOT), rifiloxifen (RAL), ICI 192,780 (ICI), genistein (GEN) (Santa Cruz Biotechnology, CA) for 24 h. Cell activity was quantitated as described previously (46). β-Galactosidase activity was determined (52) and used to normalize for individual differences in transfection efficiency. β-Galactosidase activities were similar in the 3 cell lines indicating that transfection efficiency of the bone, uterine, and mammary cells were comparable. The statistical analysis software package SAS 5.02195 (SAS Institute, Cary, NC) was used for analysis of variance (ANOVA) determinations.

**RESULTS**

### ERα and ERβ Differentially Modulate Transcription of a Reporter Plasmid Containing PR Gene Sequence in Response to Different Ligands—

The abilities of ERα and ERβ to activate transcription through EREs have been well documented (46, 48, 53, 54), but the abilities of these receptors to activate transcription through AP-1 or Sp1 sites is less clear. To better understand how ERα and ERβ regulate transcription of a complex promoter containing AP-1 and Sp1 sites in different cell environments, transient transfections were performed in bone (U2-OS), uterine (HEC-1), and mammary (SK-BR-3) cell lines using an ERα or ERβ expression vector and a reporter plasmid containing 1.5 kb of the human PR gene (−711/+817 PR TATA-CAT), which contains the −61 Sp1, +90 AP-1, +571 ERE/Sp1, and +745 AP-1 sites (Fig. 1).

When transfections were performed in the presence of an ERα expression vector, cell-specific differences in transcription were observed. When transfections were carried out in bone cells with −711/+817 PR TATA-CAT, activation was seen with all ligands except ICI (Fig. 2A). However, only E2 was able to activate transcription of −711/+817 PR TATA-CAT in uterine cells. In the mammary cell line, all ligands, including the antiestrogen ICI, induced transcription of this reporter plasmid.

When transfections were carried out with an ERβ expression vector and −711/+817 PR TATA-CAT, activation was seen in bone cells with GEN and DAI (Fig. 2D) and ICI was able to repress transcription in uterine cells. However, no changes were observed with any of the hormone treatments when ERβ was transfected into mammary cells. As a reference, transfections were also carried out with the reporter plasmid ConsERβ +10 TATA-CAT, which contains a consensus ERE upstream of a TATA box. Although transcription was increased by ERα in the presence of E2 in all three cell lines, TOT, GEN, and DAI activated transcription exclusively in bone cells (Fig. 2C). Transcription was repressed in mammary cells that had been treated with TOT or ICI.

When transfections were performed with the ERE-containing reporter plasmid and the ERβ expression vector in bone cells, activation was similar to that observed with ERα, but the level of activation was decreased (Fig. 2D). E2 activated and RAL repressed transcription in uterine cells, but no changes in

**FIG. 1. Cis elements in the human PR gene.** Schematic representation of the −61 Sp1, +90 AP-1, +571 ERE/Sp1, and +745 AP-1 sites in the −711 to +817 region of the human PR gene.

**Western Blot Analysis—** Transfected cells were harvested in TNE (40 mM Tris, pH 7.5, 1.5 mM EDTA, 140 mM NaCl), pelleted, resuspended in 250 mM Tris, and subjected to three freeze-thaw cycles to lyse cells for whole cell extracts. Nuclear extracts from untransfected U2-OS, HEC-1, and SK-BR-3 cells were prepared as described (29) in the absence of hormone. Whole cell (20 µg) or nuclear extracts (50 µg) were fractionated by SDS-PAGE and transferred to nitrocellulose membrane. Proteins were detected using goat polyclonal antibodies to Sp1 (sc-59G, respectively, Santa Cruz Biotechnology, Inc., Santa Cruz, CA), rabbit polyclonal antibodies to Fos (sc-52, Santa Cruz Biotechnology, Inc., Santa Cruz, CA), mouse monoclonal antibodies to c-Jun, ERα, ERβ (sc-45, sc-8002, and sc-8974, respectively, Santa Cruz Biotechnology, Inc., Santa Cruz, CA), or a PR monoclonal antibody that recognizes both the A and B forms of the receptor (RM-9012-S1, LabVision Corp., Fremont, CA, or MS-194-P1, NeoMarkers, Fremont, CA). Blots were probed with horseradish peroxidase-conjugated secondary antibody (Zymed Laboratories, Inc., San Francisco, CA) and the SuperSignal West Pemto Maximum Sensitivity Substrate chemiluminescent detection kit (Pierce Chemical Co.) was used to visualize the proteins as per the manufacturer’s instructions.
transcription were observed when ERβ was transfected into mammary cells.

Overall, ERα was a more potent activator of transcription than ERβ in the three cell lines tested. Interestingly, ERα produced similar transcription profiles with -711/+817 PR TATA-CAT and ConsERE +10 TATA-CAT in bone and uterine cells, but had distinctly different effects on transcription in mammary cells.

**Promoters Containing the -61 Sp1 and +571 ERE Sp1 Site Are Activated to Different Extents—**The 1.5-kb region of the PR gene used in our transfection assays contains multiple cis elements. Because it is difficult to understand how each cis element contributes to transcriptional control of this complex promoter, we examined the individual contributions of the AP-1 and Sp1 sites that reside in the 1.5-kb region of the PR gene, each of which confers estrogen responsiveness to the PR gene (29, 30, 44, 45, 55).

When transient transfection assays were carried out in bone cells with an ERα expression vector and a reporter plasmid containing the -61 Sp1 site, ERα functioned as an activator of transcription in the presence of all ligands tested except ICI (Fig. 3A). E2 activated and ICI repressed transcription in uterine cells. E2, TOT, and RAL modestly induced transcription of -61 Sp1 TATA-CAT in the mammary cells.

ERβ acted as a moderate activator of transcription in bone cells when E2 or DAI was present (Fig. 3B). Even though basal activity was higher in the uterine cells, significant induction was observed when these cells were transfected with ERβ and treated with E2. Transcription was significantly decreased by treatment of uterine cells with TOT. ERβ was unable to alter transcription of -61 Sp1 TATA-CAT in mammary cells.

ERα activated transcription of +571 ERE/Sp1 TATA-CAT in bone cells in the presence of E2, TOT, GEN, and DAI, but repressed transcription when ICI was present (Fig. 3C). Transcription was enhanced in uterine and mammary cells in the presence of E2 and diminished in uterine cells in the presence of ICI. ERβ activated transcription in the presence of E2 and GEN in bone cells, but did not affect transcription in uterine or mammary cells (Fig. 3D).

The levels of CAT activity observed with -61 Sp1 TATA-CAT were substantially greater than those observed with +571 ERE/Sp1 TATA-CAT (compare the y axis scales in Fig. 3, panels A and C with panels B and D). In addition, although E2 increased transcription in more than one cell type, the phytoestrogens GEN and DAI were only effective in activating transcription of Sp1-containing reporter plasmids in bone cells. Again ERβ was a less potent activator of transcription than ERα.

**Promoters Containing the +90 and +745 AP-1 Sites Are Activated to Different Extents—**To assess the contribution of AP-1 sites in conferring hormone responsiveness, we examined transcription mediated by reporter plasmids (+90 AP-1 TATA-CAT and +745 AP-1 TATA-CAT), which contain two different AP-1 sites that are present in the PR gene. ERα activated transcription of +90 AP-1 TATA-CAT in bone and uterine cells in the presence of E2. In contrast, transcription was unaffected by E2 treatment of mammary cells but was repressed by ICI (Fig. 4A). ERβ failed to influence transcription of +90 AP-1.
TATA-CAT in any of the three cell lines examined (Fig. 4B). When transfections were performed with +745 AP-1 TATA-CAT, ERα activated transcription in bone cells in the presence of E2 (Fig. 4C). E2 and GEN increased transcription when ERα was expressed in uterine cells. No change in transcription was observed with any of the hormones in mammary cells. ERα was unable to enhance transcription in any of the cell lines with any of the hormone treatments utilized, but acted as a repressor of transcription in bone cells in the presence of RAL and in uterine cells in the presence of TOT, RAL, and ICI (Fig. 4D).

In contrast to the high levels of transcription seen with reporter plasmids containing Sp1 sites, reporter plasmids containing AP-1 sites were significantly less transcriptionally active. Unlike the −711/+817 PR- and Sp1-containing reporter plasmids, where the highest levels of activation were most often seen in bone cells, the highest levels of transcription of AP-1-containing reporter plasmids were observed in uterine cell lines when ERα was expressed. The magnitudes of these AP-1-mediated responses were, however, less than those observed with the Sp1-containing reporter plasmids. ERβ was ineffective in activating transcription through the AP-1-containing promoters.

Different Levels of AP-1 and Sp1 Proteins Are Present in Bone, Uterine, and Mammary Cells—We were able to detect discrete differences in the abilities of ERα and ERβ to modulate transcription through AP-1 and Sp1 sites in the three cell lines examined. These differences in transcription could, in part, result from tissue-specific differences in the expression of transcription factors required for formation of an active transcription complex. To determine whether the levels of AP-1 and Sp1 proteins differed, Western analysis was performed with nuclear extracts from untransfected bone, uterine, and mammary cells. As anticipated, ERα was not detected in any of the cell lines tested and ERβ was not present in bone or mammary cells, but was detected at very low levels in uterine cells (Fig. 5). Sp1 expression was the highest in mammary cells and the lowest in uterine cells. Fos and Jun were present at lower levels in mammary cells than in bone and uterine cells. Thus, we observed cell-specific variations in transcription factors required for activation of our Sp1- and AP-1-containing reporter plasmids.

To determine whether increased expression of Sp1 or AP-1 proteins might be able to increase transcription in uterine or mammary cells, which express low levels of these AP-1 proteins, respectively, transient transfections were carried out. When a reporter plasmid containing either the −61 Sp1 (−61 Sp1 TATA-CAT) or +571 ERE/Sp1 (+571 ERE/Sp1 TATA-CAT) site from the human PR gene was transfected into the uterine cells, which express low levels of Sp1, there was a dose-dependent increase in ERα- and E2-mediated transcription (Fig. 6A). When a reporter plasmid containing the +90 AP-1 site and increasing amounts of the c-Fos and c-Jun expression vectors were transfected into mammary cells, which express low levels of these AP-1 proteins, there was no change in transcription (Fig. 6B).
These results are in agreement with previous studies in which increasing amounts of Fos and Jun were unable to enhance ER\textsuperscript{H9251}-mediated transcription of \textsuperscript{90} AP-1 TATA-CAT in U2-OS bone cells (30). Thus, the ability of ER\textsuperscript{H9251} to alter gene expression through AP-1 and Sp1 sites cannot be predicted solely by monitoring the levels of AP-1 and Sp1 proteins.

The Endogenous PR Gene Is Regulated by ER\textsuperscript{α} and ER\textsuperscript{β} in Bone Cells, but Not in Uterine or Mammary Cells—We observed distinct differences in the abilities of ER\textsuperscript{α} or ER\textsuperscript{β} expression vector to influence gene expression in bone, uterine, and mammary cells. One question of interest was whether the endogenous PR gene was actually expressed in these cells and whether ER expression might confer E2-mediated activation of the endogenous PR gene. Each of the three cell lines was transfected with an ER\textsuperscript{α} or ER\textsuperscript{β} expression vector and Western blot analysis was used to monitor the level of PR present in whole cell extracts prepared from cells that had or had not been treated with E2. Interestingly, the level of PR-B was increased when an ER\textsuperscript{α} or ER\textsuperscript{β} expression vector was transfected into bone cells and E2 was present, but we were unable to detect any difference in PR-B levels in uterine and mammary cells. These differences in PR-B expression did not result from differences in transfection efficiency because expression of a constitutively active \textsuperscript{β}-galactosidase reporter plasmid was similar in all three cell lines. No PR-A was detected in any of the three cell lines with two different antibodies that recognize both PR-A and PR-B. However, both PR-A and PR-B were detected in nuclear extracts from MCF-7 cells that had been treated with E2 for 24 h (data not shown). These findings suggest that expression of the endogenous PR gene in uterine and mammary cells may require additional proteins for enhanced transcription, that the PR gene in uterine and mammary cells is regulated by a different set of transcription factors than are utilized in bone cells, or that the endogenous PR gene is less accessible in the uterine and mammary cell lines utilized than in the bone cell line.

DISCUSSION

Transcriptional Modulation by ER\textsuperscript{α} and -\textsuperscript{β}—A number of studies have demonstrated that ER\textsuperscript{α} is a more potent activator of ERE-containing reporter plasmids than ER\textsuperscript{β} (46, 53, 56), but the abilities of these two receptors to activate transcription through AP-1 and Sp1 sites has been less clear. The compiled
transfection data in Table I provides a comparative analysis of the abilities of these two receptors to regulate transcription of promoters containing AP-1 and/or Sp1 sites and allows us to make a number of observations. First, ERα was a more potent activator of AP-1- and Sp1-driven transcription in the bone, uterine, and mammary cells utilized than ERβ. Second, ERα responded to a larger repertoire of hormonal ligands than ERβ. Third, ERα was far more effective in increasing transcription of a complex promoter containing multiple AP-1 and Sp1 sites (−711/+817 TATA-CAT) than ERβ. Fourth, ERα was very effective in activating transcription of Sp1-containing reporter plasmids in bone cells, especially −61 Sp1 TATA-CAT, but ERβ only modestly increased transcription of reporter plasmids containing Sp1 sites in these cells. Finally, although ERα activated transcription of AP-1-containing reporter plasmids, ERβ was unable to activate transcription through either of the AP-1 sites tested. Clearly ERα is a more potent transcriptional activator of the five AP-1- and Sp1-containing promoters used in this study than ERβ.

Our results with Sp1-containing promoters are in agreement with an earlier study, which demonstrated that ERα was more effective in activating transcription at a Sp1-containing promoter than ERβ in MCF-7 and MDA-MB-231 breast cancer cells (57). However, our findings with the AP-1-containing promoters contrast with those of Paech et al. (58), who reported that ERβ activates transcription of a reporter plasmid containing the collagenase AP-1 site in uterine (Ishikawa) and mammary (MCF-7) cells in the presence of TOT, RAL, and ICI. In fact, we observed ERβ-induced transcriptional repression rather than activation of +745 AP-1 TATA-CAT with TOT, RAL, and ICI in uterine cells and with RAL in bone cells. It is difficult to reconcile these apparent contradictions, but it does seem possible that differences in AP-1 sequence, nucleotide sequence flanking the AP-1 site, promoter construction, ERα expression vectors (HEO versus HEGO), or cellular context could contribute to the differences observed. We did note that in an earlier study by the same group, TOT did not increase transcription of a promoter containing the collagenase AP-1 site in MCF-7 or ZR75 mammary cell lines (27).

Modulation of Gene Expression by AP-1 and Sp1 Sites—We have assessed the transcription elicited by a composite, naturally occurring gene sequence and four individual cis elements. The interactions of these cis elements with their corresponding trans-acting factors have been characterized in detail using in vitro and in vivo approaches (29, 30, 44, 45, 55). These studies have shown that Sp1 binds to both of the tandem Sp1 sites in the −61 Sp1 and +571 ERE/Sp1 sites and that ERα enhances Sp1 binding to these sites and binds directly to the ERE half-site in the +571 ERE/Sp1 site (44, 45). Additional studies have shown that Fos and Jun bind as heterodimers to the +90 and +745 AP-1 sites and that ERα enhances binding of the AP-1 proteins to these sites in vitro and in vivo (29, 30). Thus, we know a good deal about the binding of Fos, Jun, and Sp1 to their respective recognition sequences. However, the relative contributions of these individual cis elements in regulating transcription and the transcriptional response of AP-1 and Sp1 sites with slightly varied DNA sequence had not been addressed.

The −711/+817 region of the PR gene contains the −61 Sp1, +90 AP-1, +571 ERE/Sp1, and +745 AP-1 sites, all of which confer estrogen responsiveness to the PR gene (29, 30, 44, 45, 55). A reporter plasmid containing this 1.5-kb region of the PR gene was significantly more hormone responsive than any of the reporter plasmids containing an AP-1 or Sp1 site alone (Table I). When each of the four cis elements was tested individually, the two Sp1 sites were significantly more potent transcriptional enhancers than either of the AP-1 sites. The −61 Sp1 site, which is comprised of two adjacent Sp1 sites, activated transcription to higher levels than any of the other cis elements tested (Figs. 2–4). The abilities of the −61 Sp1 and +571 ERE/Sp1 sites to activate transcription to different extents suggest that individual Sp1 sites have distinct capacities to activate transcription and are not functionally equivalent. Interestingly, previous studies in our laboratory demonstrated that the +571 ERE/Sp1 site is a more potent transcriptional enhancer when the ERE half-site is mutated (55) supporting the idea that the DNA sequence flanking a cis element can alter the transcriptional response. The activity elicited by the −61 Sp1 site in response to different hormones most closely paralleled the activity of the 1.5-kb region of the PR gene. However, the −61 Sp1 site transcriptional profile was not identical to that of the larger PR DNA sequence reinforcing the idea that it is the combined contribution of multiple cis elements that regulate PR gene expression and not any single cis element alone.

The AP-1 sites alone were nearly transcriptionally inert. However, we know that mutation of either one of these sites in the context of the 1.5-kb promoter causes dramatic changes in transactivation (29, 30). Thus, even though the AP-1 sites alone are very modest transcriptional enhancers individually, they...
still contribute to the overall hormone responsiveness of the PR gene. Furthermore, despite the fact that the +90 and +745 AP-1 sites have essentially identical nucleotide sequence, they each had distinct responses to hormone treatments again suggesting that DNA sequences flanking the AP-1 sites influence transactivation. The ability of flanking DNA sequence to alter AP-1-driven activation has been reported previously (31).

If one compares the promoter activities of −61 Sp1 TATA-CAT in the three cell lines tested, this reporter plasmid elicited significantly higher levels of transcription than −711/+817 PR TATA-CAT in the absence and presence of hormone (Figs. 2 and 3). These results suggest that other cis elements in the 1.5-kb promoter must be involved in limiting transcription in the absence and presence of hormone. In fact, we have identified an ERE half-site in the +571 ERE/Sp1 site and another ERE half-site adjacent to the +745 AP-1 site that limit basal and E2-induced transcription. If either of these ERE half-sites is mutated, transcription is greatly increased in the absence and presence of E2 (30, 55).

**Ligand-specific Modulation through AP-1 and Sp1 Sites—**

The battery of hormones utilized in our study selectively modulated transcription of AP-1 and Sp1-containing promoters. When cells were transfected with ERα, E2 was the most potent of all the ligands tested. E2 significantly increased transcription through the composite PR gene region and through each of the individual cis elements in all three cell lines tested with two exceptions: E2 was unable to alter transcription of the +90 and +745 AP-1-containing reporter plasmids in mammary cells. The selective estrogen receptor modulators TOT and RAL had more restricted effects on transcription than E2. Whereas TOT and RAL were effective in regulating transcription through the −61 Sp1 site in bone and mammary cells, they failed to influence AP-1-mediated transcription. As anticipated, the inhibitory effect of ICI on transcription was evident with AP-1 and Sp1-containing reporter plasmids. Our investigations suggest that the ability of the phytoestrogens GEN and DAI to increase transcription of PR gene expression in bone and mammary cells is mediated primarily through the −61 Sp1 and +571 ERE/Sp1 sites. ERβ was significantly less responsive to hormone treatments than ERα. Interestingly, it does appear that the effects of GEN and DAI on PR gene expression may be mediated primarily through the +571 ERE/Sp1 site and the −61 Sp1 site.

**Importance of Cell Context in Regulating Transcription—**

Each of the cell lines utilized in this study had distinct characteristics and responsiveness to hormone stimulation. One of the more striking features of our transfection data was the high basal expression of our reporter plasmids in uterine cells in the absence of hormone (Figs. 2–4). Bone cells were the most transcriptionally active of the cell lines tested and the endogenous PR gene was enhanced by E2 treatment of these cells when either ERα or ERβ was expressed (Fig. 7). The mammary cell line was typically the least transcriptionally responsive.

In an attempt to determine whether enhanced expression of Sp1 or AP-1 proteins influenced transcription, we transfected uterine cells, which express low levels of Sp1, with an Sp1 expression vector. In fact, increasing the expression of Sp1 significantly enhanced transcription of −61 Sp1 TATA-CAT and +571 ERE/Sp1 TATA-CAT. Overexpression of Fos/Jun, however, failed to enhance transcription of +90 AP-1 TATA-CAT in mammary cells, which express low levels of these AP-1 proteins.

Taken together, our findings suggest that the expression of a single protein is not necessarily a good predictor of transcriptional response in a cell line and underscores the complex nature of transcriptional control, which relies on the combined contributions of multiple cis elements and trans-acting factors. Furthermore, transcriptional processes almost certainly rely on as yet unidentified proteins. The identification of novel

**TABLE I**

Compiled data from transient transfection studies

| Ligand | ERα | ERβ | Uterine | Bone | Mammary |
|--------|-----|-----|---------|------|---------|
| E2     | 52.38 | 3.35 | 1.174 | 2.55 | 1.65 | 1.88 | 7.25 | 2.17 | 1.47 | 3.11 | 1.88 |
| TOT    | 6.45  | 3.42 | 2.23  | 2.73 | 2.91 | 3.36 | 3.48 | 2.51 | 3.17 | 2.68 | 1.37 |
| ICI    | 2.11  | 1.99 | 2.1  | -1.64 | -1.75 | -2.33 | -1.45 | -1.37 | -1.34 |
| GEN    | 3.33  | 3.25 | 1.69  | 3.94 | 1.97 | 1.76 | 1.76 | 2.01 | 2.01 | 2.01 | 2.01 |
| DAI    | 3.35  | 3.30 | 1.70  | 4.42 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |

**FIG. 7. Expression of the endogenous PR gene in bone, uterine, and mammary cells.** U2-OS (bone), HEC-1 (uterine), or SK-BR3 (mammary) cells were transfected with ERα or ERβ expression vectors. Whole cell extracts (20 μg) prepared from transfected cells were run on SDS-PAGE gels and transferred to nitrocellulose membrane. Western blot analysis was performed to monitor PR protein levels.
coregulatory proteins associated with AP-1 and Sp1 sites and involved in regulating hormone responsiveness is currently under investigation in our laboratory.

**Regulation of AP-1- and Sp1-containing Genes**—Although other laboratories have examined the abilities of ERα and/or ERβ to regulate transcription of Sp1- and AP-1-containing promoters, this is the first study to undertake a comparative analysis of the effectiveness of these sites using side-by-side comparisons. Our studies provide detailed information about the role of four naturally occurring AP-1 and Sp1 sites in regulating PR gene expression and provide new insight to better understand how other estrogen-responsive genes containing AP-1 and/or Sp1 sites are regulated in response to hormonal ligands.

**Acknowledgment**—We thank Wendy Smith for technical assistance.

**REFERENCES**

1. Korach, K. S. (1994) *Science* **266**, 1524–1527
2. Hess, R. A., Gist, D. H., Bunick, D., Lubahn, D. B., Farrell, A., Bahr, J., Cooke, P. S., and Greene, G. L. (1997) *J. Androl.* **18**, 602–611
3. Couse, J. F., Curtis Hewitt, S., and Korach, K. S. (2000) *J. Steroid Biochem. Mol. Biol.* **74**, 287–296
4. Couse, J. E., Mahato, D., Eddy, E. M., and Korach, K. S. (2001) *Reprod. Fertil. Dev.* **13**, 211–219
5. Subbaih, M. T. (1998) *Proc. Soc. Exp. Biol. Med.* **217**, 23–29
6. Mendelsohn, M. E., and Karas, R. H. (1994) *Curr. Opin. Cardiol.* **9**, 619–626
7. Toran-Allerand, C. D. (1996) *Dev. Neurosci.* **18**, 36–48
8. Turner, R. T., Rigs, B. L., and Spielberg, T. C. (1994) *Endocr. Res.* **15**, 275–300
9. Jordan, V. C., and Curtis Hewitt, S. (1995) *Cancer Res.* **55**, 4999–5006
10. Goyette, D. R., Allred, K. F., Allred, C. D., and Helferich, W. G. (2002) *Cell Biol.* **183**, 2151–2158
11. Delmas, P. D., Bjarnason, N. H., Mitlas, B. H., Ravoux, A.-C., Shah, A. S., Hirt, W. J., Draper, M., and Christiansen, C. (1997) *Ann. N. Y. Acad. Sci.* **869**, 146–151
12. Loven, M. A., Wood, J. A., and Nardulli, A. M. (2000) *Mol. Endocrinol.* **14**, 972–985
13. Leven, M. A., Wood, J. A., and Nardulli, A. M. (2001) *Mol. Cell. Endocrinol.* **181**, 151–163
14. Reese, J. C., and Nardulli, B. S. (1991) *Nucl. Acids Res.* **19**, 6595–6602
15. McInerney, E. M., Weis, K. E., Sun, J., Mosselman, S., and Katzenellenbogen, B. S. (1998) *Endocrinology* **139**, 4513–4522
16. Hu, C. D., Chinenov, Y., and Korppola, T. K. (2002) *Mol. Cell* **9**, 789–798
17. Chang, T. C., Nardulli, A. M., Law, D., and Shapiro, D. J. (1999) *Endocrinology* **139**, 346–354
18. Nardulli, A. M., Romine, L. E., Carpe, C., Greene, G. L., and Rainbath, B. (1996) *Mol. Endocrinol.* **10**, 694–704
19. Cowley, S. M., Hoare, S., Mosselman, S., and Parker, M. G. (1997) *J. Biol. Chem.* **272**, 18583–18582
20. Tremblay, A., Tremblay, G. B., Labrie, C., Labrie, F., and Giguere, V. (1998) *Endocrinology* **139**, 111–118
21. Petz, L. N., Ziegler, Y., Stenberg, A., and Giguere, V. (2004) *J. Steroid Biochem. Mol. Biol.* **88**, 115–122
22. Benecke, A., Chambon, P., and Gromemeyer, H. (2000) *EMBO Rep.* **1**, 151–157
23. Saville, B., Wormke, M., Wang, F., Nguyen, T., Enmark, E., Kupfer, G., and Gustafsson, J. A., and Safe, S. (2000) *J. Biol. Chem.* **275**, 5379–5387
24. Pasch, K., Webb, P., Kupfer, G. G., Nilsson, S., Gustafsson, J. A., and Scanlan, T. (1997) *Science* **277**, 1508–1510

---

**Regulation of AP-1- and Sp1-containing Promoters**
Cell- and Ligand-specific Regulation of Promoters Containing Activator Protein-1 and Sp1 Sites by Estrogen Receptors α and β
Jennifer R. Schultz, Larry N. Petz and Ann M. Nardulli

J. Biol. Chem. 2005, 280:347-354.
doi: 10.1074/jbc.M407879200 originally published online October 26, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M407879200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 58 references, 12 of which can be accessed free at
http://www.jbc.org/content/280/1/347.full.html#ref-list-1