AF-2 of Estrogen Receptor
p300 Mediates Functional Synergism between AF-1 and AF-2 of Estrogen Receptor α and β by Interacting Directly with the N-terminal A/B Domains

Received for publication, January 3, 2000, and in revised form, February 24, 2000
Published, JBC Papers in Press, March 15, 2000, DOI 10.1074/jbc.M000042200

Yoko Kobayashi‡, Takuya Kitamoto‡, Yoshikazu Masuhiro‡, Michiko Watanabe‡, Toshiya Kase‡, Daniel Metzger‡, Junn Yanagisawa‡,‡, and Shigeaki Kato‡‡

From the ‡Institute of Molecular and Cellular Biosciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-0032, Japan, §Institut de Genetique et de Biologie Moleculaire et Cellulaire/ CNRS/INSERM/ULP College de France, BP 163, 67404 Illkirch Cedex, C.U. de Strasbourg, France, andCREST, Japan Science and Technology, Kawaguchi, Saitama 332-0012, Japan

Estrogen receptor (ER) α and β mediate estrogen actions in target cells through transcriptional control of target gene expression. For 17β-estradiol-induced transactivation, the N-terminal A/B domain (AF-1) and the C-terminal E/F domain (AF-2) of ERs are required. Ligand binding is considered to induce functional synergism between AF-1 and AF-2, but the molecular mechanism remains unknown. To clarify this synergism, we studied the role of reported AF-2 coactivators, p300/CREB binding protein, steroid receptor coactivator-1/transcriptional intermediary factor-2 (SRC-1/TIF2) family proteins and thyroid hormone receptor-associated protein-220/vitamin D3 receptor-interacting protein-205-(TRAP220/DRIP205) on the AF-1 activity in terms of synergism with the AF-2 function. We found that neither any of the SRC-1/TIF2 family coactivators nor TRAP220/DRIP205 is potent, whereas p300 potentiates the AF-1 function of both human ERα and human ERβ. Direct interactions of p300 with the A/B domains of ERα and ERβ were observed in an in vitro glutathione S-transferase pull-down assay in accordance with the interactions in yeast and mammalian two-hybrid assays. Furthermore, mutations in the p300 binding sites (56–72 amino acids in ERα and 62–72 amino acids in ERβ) in the A/B domains caused a reduction in ligand-induced transactivation functions of both ERα and ERβ. Thus, these findings indicate that ligand-induced functional synergism between AF-1 and AF-2 is mediated through p300 by its direct binding to the A/B regions of ERα and ERβ.

These findings indicate that, during the ligand-induced transactivation, a coactivator complex is recruited, forming a higher complex with the nuclear receptor to achieve activation and repression of transcription. Several coactivators directly interacting with the E/F domain in a ligand-dependent manner have been identified, including the SRC-1/TIF2 (20–22) and p300/CBP (23–26) families, TIF1 (27, 28), ARA70 (29), PGC1 (30), and many others (31, 32, 33). More recently, a coactivator complex TRAP/DRIP containing none of the reported coactivators has been identified, and one of the components, TRAP220/DRIP205 is shown to directly interact with the E/F domain of some nuclear receptors including ERα in a ligand-dependent way (34, 35). Thus, such AF-2 coactivators have been well studied, whereas little is known about the AF-1 coactivator(s).

The present study was thus undertaken to clarify the ligand-induced functional synergism between AF-1 and AF-2 in ERα as well as ERβ, with attention paid to the actions of known AF-2 coactivators in the AF-1. Consequently, we found that the core domains for both the ERα and ERβ AF-1 activities in the A/B domains are indistinguishable from those responsible for the functional synergism with each of the AF-2s. Though
known coactivators (SRC-1, TIF2, and AIB1) enhanced the AF-2 of ERα as well as ERβ, neither of them nor TRAP220/DRIP205 potentiated the AF-1s of ERα and ERβ. However, consistent with the transcriptional potentiation by p300, direct interaction with the ERα and ERβ A/B domains was detected only in p300 in vitro. Furthermore, the ligand-induced transactivation functions of ERα and ERβ were impaired by the mutations of the p300 binding sites in their A/B domains. Thus, the present study indicates that p300 mediates, at least in part, the functional synergism between AF-1 and AF-2 through its direct binding of the A/B regions of ERα and ERβ.

**EXPERIMENTAL PROCEDURES**

**Plasmids—**The mammalian expression vectors for human ERα-HEG0 (8), HE15 (7), and HEG19 (9) have been described previously. Full-length human ERβ cDNA (1) was isolated from the HeLa cDNA library, and the pSG5 mammalian expression vector was constructed. The amino acid residues present in each of the hERβ deletion mutants are shown in Fig. 1A. The GAL-ER and VP16-ER mutant expression plasmids containing the GAL4 DNA binding domain and the VP16 activation domain were constructed by subcloning cDNA fragments into the appropriate sites of the multicloning regions in pM and pVP (CLONTECH). The GAL-ER mutants (VP16-ER and GAL4-ER) were deleted in 534–595 aa of the hERs E/F domain and 432–477 aa of the hERβ E/F domain. SRC-1, TIF2, AIB1, and TRAP220 cDNAs were also isolated from the HeLa cDNA library, and the expression vectors were constructed by introducing the cDNAs into pcDNA3 (Invitrogen) (36). The reporter plasmid ER response element (ERE)-G-chloramphenicol acetyltransferase (CAT) contains an ERE, β-globin promoter, and CAT gene, and 17M2G-CAT contains the GAL4 upstream activation sequence (17-mer (21)), β-globin promoter, and CAT gene. Glutathione S-transferase (GST)-hERαB and -αB mutants (GST-αM1, -αM2, -αM3, -αM4, -αM5, -αM6, -αM7, -αM8, -αM9, -αM10, -αM11, -αM12, or -αM13) and GST-αE/F, -βA/B, and -βA/B mutants (GST-βM1, -βM2, -βM3, -βM4, -βM5, -βM6, -βM7, -βM8, -βM9, -βM10, or -βM11) and GST-βE/F fusion proteins were prepared by amplification of each sequence by standard PCR methods, and the resulting fragments were cloned in frame into pGEX-4T1 (Amersham Pharmacia Biotech) using appropriate restriction sites.

**Cell Culture and CAT Assay—**COS-1 cells were maintained in Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) without phenol red and supplemented with 5% fetal bovine serum, which had been stripped with dextran-coated charcoal in 10-cm dishes. At 40–60% confluency, cells were transfected by calcium phosphate with 2 μg of ERE-G-CAT or 17M2G-CAT reporter plasmid, either 0.3 μg of ER expression vector, 1 μg of GAL4-fused ER expression vector, or 3 μg of coactivator expression vector, along with 3 μg of transfection indicator pCH110 (Amersham Pharmacia Biotech), and Bluescribe M13 (Stratagene) was used as carrier DNA to adjust the total amount of DNA to 20 μg (37). After a 24-h incubation in medium containing the precipitated DNA, the cells were washed with fresh medium and continued to grow for an additional 24 h with or without 10−8 M E2. Cell extracts were prepared by freeze-thawing and assayed for CAT activity after normalization for β-galactosidase activity (37). Results from CAT assays were analyzed by TLC, and the TLC plate was quantified using an image analyzer (BAS1500, Fuji Film, Tokyo, Japan) and shown as mean ± standard deviations calculated from the three independent experiments.

**Mammalian Two-hybrid Assay—**COS-1 cells were maintained as described above for the CAT assay. The cells were transfected with 2 μg of 17M2G-CAT reporter plasmid, either 1 μg of GAL4- or VP16-fused ER expression vector or 3 μg of coactivator expression vector, along with 3 μg of pCH110 (Amersham Pharmacia Biotech), and Bluescribe M13 (Stratagene) was used as carrier DNA to adjust the total amount of DNA to 20 μg (32). After a 24-h incubation in medium containing the precipitated DNA, the cells were washed with fresh medium and continued to grow for an additional 24 h with or without 10−8 M E2. CAT activity was measured and shown as described above.

**GST Pull-Down Assay—**The GST fusion protein or GST alone was expressed in Escherichia coli as described (32), and the expression of proteins of the predicted size was then monitored by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. For GST pull-down assays, bacterially expressed GST fusion proteins or GST bound to glutathione-Sepharose-4B beads (Amersham Pharmacia Biotech) and incubated with [35S]-labeled proteins were expressed by in vitro translation using the TNT-coupled transcription-translation system, with conditions as described by the manufacturer (Promega). After 2 h of incubation, free proteins were washed away from the beads with NET-N− buffer (150 mM NaCl, 1 mM EDTA, 20 mM Tris-HCl, pH 7.5, 0.5% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, and 1 mM dithiothreitol). Specifically bound proteins were eluted by boiling in SDS sample buffer and analyzed by 10% SDS-polyacrylamide gel electrophoresis. After electrophoresis, radiolabeled proteins were visualized using an image analyzer (BAS1500, Fuji Film, Tokyo, Japan).

**Western Blotting Analysis—**COS-1 cells were transfected with the indicated plasmids and lysed in TNE buffer (10 mM Tris-HCl, pH 7.8, 1% Nonidet P-40, 150 mM NaCl, 1 mM EDTA). Proteins were separated by 10% SDS-polyacrylamide gel electrophoresis, transferred onto polyvinylidene difluoride membranes (Bio-Rad), and detected with anti-GAL4 DBD antibody (Santa Cruz Biotechnology) and anti-rabbit IgG conjugated with horseradish peroxidase (Santa Cruz Biotechnology) (32).

**Fig. 1. Ligand-induced and functional interaction between the A/B and E/F domains in hERα and hERβ in vitro.** E2-induced functional interaction between the A/B and E/F domain of hERα (A), and hERβ (B), and also interaction between domains derived from different ER subtypes (C). Interactions between A/B and E/F domains of ERs were examined in the mammalian two-hybrid system in the presence (closed columns) or absence (open columns) of E2 (10−8 M) as described under "Experimental Procedures." COS-1 cells were transfected with 3 μg of 17M2G-CAT, along with 1 μg of expression vector containing either A/B or E/F domains of ERs fused to the GAL4 DNA binding domain (GAL4-aA/B, GAL4-aE/F, GAL4-aαA/B, or GAL4-βE/F), and 1 μg of the expression vector bearing either the A/B or E/F domain of ERs fused to the VP16 activation domain (VP16-aA/B, VP16-aE/F, VP16-βA/B, or VP16-βE/F). CAT activities were normalized relative to the β-galactosidase activities expressed by the pCH110 internal control vector and are reported as mean ± S.D. calculated from the three independent experiments.
GAL4- and p300 bound directly to A/B domains of ERs. The ligand-induced interaction between A/B and E/F domains is indirect. A/B and E/F or VP16 transactivation domains were expressed in COS-1 cells and were measured and are shown as mean ± S.D. calculated from the three independent experiments.

**RESULTS**

Functional and Ligand-induced Interaction of the A/B Domain with the E/F Domain Is Indirect in hERαand hERβ—For the ligand-induced transactivation in ERs, two domains in the N-terminal A/B and the C-terminal E/F domain act synergistically to stimulate transcription. However, the molecular mechanism of this synergism is largely unknown. Therefore, we first tested in vivo the interaction between the A/B and E/F domains in hERα and hERβ by a mammalian two-hybrid assay with the hER deletion mutants fused to either the GAL4 DBD or VP16 transactivation domain (32). As expected from previous reports (18, 19), ligand-induced interaction between the A/B and E/F domains in hERα was observed (Fig. 1A). Though the hERα A/B domain did not form a homodimer, it interacted with the E/F domain in a ligand-dependent way. Similar interactions between the two domains were detected also in hERβ (Fig. 1B). Interestingly, ligand-induced interactions between the two domains derived from different ER subtypes (α-β) were also detected (Fig. 1C). Thus, the properties of the ligand-induced interactions in the A/B-E/F and the E/F-E/F domains were assessed.

**Fig. 2.** Regions of interaction in the A/B domains for ligand-bound E/F domains in hERα and hERβ*in vivo.* The transactivation regions in A/B domains are necessary for the ligand-induced interaction between A/B and E/F domains. Interactions between truncated A/B domain mutants and E/F domains were examined in the mammalian two-hybrid system in the presence (closed columns) or absence (open columns) of E2 (10−8 M). COS-1 cells were transfected with 3 μg of 17M2G-CAT, along with either 1 μg of GAL4-αA/B, GAL4-αA/βB mutants (GAL4-αM1, -αM2, -αM3, -αM4, -αM5, -αM6, -αM7, -αM8, -αM9, -αM10, or -αM11, -αM12, or -αM13), GAL4-βA/B, or GAL4-βA/B mutants (GAL4-βM1, -βM2, -βM3, -βM4, -βM5, -βM6, -βM7, -βM8, -βM9, -βM10, or -βM11), with or without 1 μg of VP16-αE/F or VP16-βE/F. CAT activities were measured and are shown as mean ± S.D. calculated from the three independent experiments.

**Fig. 3.** p300 directly interacts with the A/B domains in hERα and hERβ and enhances the AF-1 activities of hERα and hERβ. A, ligand-induced interaction between A/B and E/F domains is indirect and p300 bound directly to A/B domains of ERs *in vitro.* GST pull-down assay was performed as described under “Experimental Procedures.” GST, GST-ERαA/B, GST-ERβA/B, GST-ERαE/F, and GST-ERβE/F were expressed in *E. coli* and immobilized on glutathione-Sepharose beads. *In vitro* translated ERαA/B, ERβA/B, SRC-1, TIF2, AIB1, p300, or TRAP220/DRIP205 was incubated with glutathione-Sepharose beads loaded with GST, GST-ERαA/B, GST-ERβA/B, GST-ERαE/F, or GST-ERβE/F in the presence or absence of E2 (10−6 M). The bound proteins were subjected to SDS-polyacrylamide gel electrophoresis followed by autoradiography. B, effects of coactivators on activities of AF-1 of ERs: p300 enhances the AF-1 activities of hERα and hERβ. COS-1 cells were transfected with 3 μg of 17M2G-CAT, together with either 0.3 μg of HE15 (hERα A-C domain) or HEJ15 (hERβ A-C domain), and 3 μg of pcDNA3-SRC-1, pcDNA3-TIF2, pcDNA3-AIB1, or pCMVβ-p300 in the absence of E2. C, p300 interacts with the A/B domains of ERs *in vivo.* Interactions between either GAL4-αA/B or GAL4-βA/B and coactivators were examined in the mammalian two-hybrid system. COS-1 cells were transfected with 3 μg of 17M2G-CAT, along with either 1 μg of GAL4-αA/B or GAL4-βA/B, and 3 μg of VP16-SRC-1, VP16-TIF2, VP16-AIB1, VP16-p300, or VP16-TRAP220. The CAT assay was performed as described under “Experimental Procedures,” and results are reported as mean ± S.D. calculated from the three independent experiments.
are indistinguishable between hERα and hERβ. We then tried to delineate the regions of ligand-induced interaction of the A/B domain for the E/F domain with a series of A/B domain deletion mutants fused to a yeast GAL4 DBD. As shown in Fig. 2A, the interaction regions were mapped to the central region (aa 56–127) of the hERα A/B domain and to the N-terminal region (aa 1–62) of the hERβ A/B domain. Interestingly, these regions almost overlapped with the core regions for the full activities of the AF-1s of hERα and hERβ, though the AF-1 activity of hERβ is half that of hERα (Fig. 2A), as expected from previous reports (2, 12). We confirmed that the expression levels of the A/B domain deletion mutants are almost the same by a Western blot analysis with an antibody for GAL4 DBD, when the cell extracts were normalized to the transfection efficiency (data not shown).

To test whether the observed in vivo interactions of the A/B domain with the ligand-bound E/F domain of hERα and hERβ are physically direct or indirect, we applied a GST pull-down assay with bacterially expressed chimeric hER proteins fused with GST. Monitoring the properties of ligand-induced direct interactions of the E/F domains with coactivators, we chose a nuclear receptor coactivator, SRC-1, and consequently SRC-1 was found to associate with both the hERα and hERβ E/F domains in a ligand-dependent manner (Fig. 3A, lanes 5–8) as reported (12, 20). However, under this condition, physical interaction between the A/B and E/F domains was detected in neither hERα nor hERβ even in the presence of ligand (Fig. 3A). Thus, these findings indicate that the ligand-induced interaction of the A/B domain with the E/F domain in hERα and hERβ is indirect and possibly mediated through a factor bridging the two domains.

p300 Acts as a Coactivator for the AF-1 Activities of hERα and hERβ—To test this idea, we studied whether the well characterized AF-2 coactivators directly interacting with the E/F domains of various nuclear receptors potentiate the AF-1 activities of hERα and hERβ. For this study, all three 160-kDa coactivators, SRC-1, TIF2, and AIB1, and p300 were used. All four coactivators potentiated the ligand-induced transactivities of the full-length hERα and hERβ (data not shown), as we expected from previous reports (21, 22, 23). Such potentions by these coactivators were also confirmed for the AF-2 activities of hERα and hERβ (data not shown), as reported (22, 23).

None of the 160-kDa coactivators potentiated the hERα and hERβ AF-1 activities in the ER A-C domains (HE15, ERα A-C domain and HEβ15, ERβ A-C domain) (Fig. 3B), whereas p300 was clearly potent (Fig. 3B).

p300 Mediates the Ligand-induced Interactions between the A/B Domain and the E/F Domain in hERα and hERβ—As p300 appeared to act as a coactivator also for the AF-1s of hERα and hERβ, we examined if p300 directly associates with the
A/B domain in vitro and in vivo. The GST pull-down assay clearly showed interactions of p300 with both the hERα and hERβ A/B domains in vitro, whereas none of the SRC-1/TIF2 family proteins were able to bind (Fig. 3A, lanes 3 and 4). TRAP220/DRIP205 showed no interaction (Fig. 3A), reflecting its inability to potentiate the AF-1s (data not shown). Consistent with these results, chimeric hERα and hERβ A/B domains fused to the GAL4 DBD exhibited in vivo interactions with p300, but not with the SRC-1/TIF2 family proteins (Fig. 3C). The binding sites of the A/B domains for p300 in vivo were further mapped to 16 aa residues (ERα aa 56–72) and 72 aa residues (ERβ 1–72) (Fig. 4, A and B). Though the p300 binding site of hERα in vivo was essential for direct binding of p300 in vitro (Fig. 4C, upper panel), the p300 binding to the hERβ A/B domain in vivo (Fig. 4C) required more region than tested in vitro (only 10 amino acids (62–72) are required for in vitro binding), suggesting that the N-terminal region (1–62 aa) contributes a stable interaction of the ERβ A/B domain with p300 in vivo. Note that the expression levels of the chimeric ER mutants with GAL4 DBD were confirmed at almost the same levels when estimated by Western blot analysis (data not shown).

Thus, as p300 is able to bind to the A/B domains in both hERα and hERβ, we next tested whether p300 enhances the ligand-induced interactions between the A/B and E/F domains in vivo, as observed in Fig. 1. The mammalian two-hybrid

Fig. 5. p300 mediates the ligand-induced interactions between the A/B domain and E/F domain in hERα and hERβ. A, p300 bound directly to A/B domains and E/F domains of ERs in vitro. GST pull-down assay was performed as described under “Experimental Procedures.” Either in vitro translated hERα/A/B, hERα/A/B, or p300 was incubated with either GST, GST-ERα/E/F, or GST-ERβ/E/F immobilized to glutathione-Sepharose beads in the presence or absence of E2 (10⁻⁶ m). The bound proteins were subjected to SDS-polyacrylamide gel electrophoresis followed by autoradiography. B, p300 stabilized the interaction between A/B and E/F domains of ERs. Effects of p300 on the interactions between GAL4-fused A/B domains and VP16-fused E/F domains were examined in the mammalian two-hybrid system in the presence (closed columns) or absence (open columns) of E2 (10⁻⁸ m). COS-1 cells were transfected with 3 μg of 17M2G-CAT, along with 1 μg each of the expression vectors of GAL4-A/B and VP16-E/F mutants with or without 3 μg of pCMVβ-p300. CAT activities were measured and are shown as mean ± S.D. calculated from the three independent experiments.

Fig. 6. Lack of p300 binding sites in A/B domains impaired the ligand-induced transactivation in hERα and hERβ. A, schematic presentation of hERα and hERβ deletion mutants. In the upper panel, the A-F domains and the corresponding AF-1 and AF-2 regions are illustrated. ERβ deletion mutants containing the A-C domain or the C-F domain are designated as HEβ15 and HEβ19, respectively, as previously reported for ERα deletion mutants (7). ΔERα-1 and ΔERβ-1 are the mutants lacking the p300 binding sites in hERα and hERβ A/B domains. The numbers of aa residues are also shown. B, direct binding of p300 to the A/B domains mediates the ligand-induced transactivation in hERα and hERβ. COS-1 cells were transfected with 3 μg of ERE-G-CAT, together with 0.3 μg of either HE15, HEG19, HEG0, ΔERα-1, ΔERβ-1, HEβ15, HEβ19, HEβ0, or ΔERβ-1 in the presence (closed columns) or absence (open columns) of E2 (10⁻⁸ m). CAT activities were measured and are shown as mean ± S.D. calculated from the three independent experiments.
system revealed that the overexpression of p300 potentiates the ligand-induced interactions (Fig. 5B). The interactions between the A/B and E/F domains were impaired when the p300 binding to the E/F domains is abolished by deleting the AF-2 cores (534–595 aa or hERα (VP16-ERαAD) and 432–477 aa of hERβ (VP16-ERβΔAD)) (23) (see Fig. 5B). Notably, these ligand-induced interactions between two domains were further detected in vitro only in the presence of p300 (Fig. 5A, lanes 10 and 15).

Lack of p300 Binding Sites in the A/B Domains Impaired the Ligand-induced Transactivation Function of hERα and hERβ—To test an idea that p300 functionally bridges the A/B domain and the E/F domain in ERα and ERβ, we made deletion mutants lacking the p300 binding sites (∆ERα-1 and ∆ERβ-1 as illustrated in Fig. 6A). As shown in Fig. 6B, the lack of binding sites caused a clear reduction in the ligand-induced transactivation in hERα and hERβ. Thus, it is most likely that the p300 action of AF-1 by its direct binding contributes to the ligand-induced transactivation function of ERα and ERβ in whole receptor. Taken together, these findings indicate that p300 mediates the ligand-induced interactions between the A/B and E/F domains in hERα and hERβ by its direct binding to the A/B domains.

DISCUSSION

The tissue-specific activity of hERα is considered to play a significant role in the actions of estrogen. Especially tamoxifen, an estrogen partial agonist, is considered to exert its tissue-specific actions through activating AF-1 and inhibiting AF-2 in hERα (9, 40). Therefore, it is of great interest to identify the cofactors responsible for the activities of the ER(s) AF-1, in terms of synergistic function with AF-2. In the present study, we identified the AF-1 activity in the A/B region of hERβ, though it was weaker than that in the hERα A/B domain in COS-1 cells. As the poor activity of hERβ AF-1 was seen in other cell types, such as HeLa cells and MCF-7 cells (data not shown), it is likely that in response to E2, the AF-1 activity contributes less than the AF-2 activity of ERβ as in ERα in most target cells in accordance with previous reports (2, 12). However, it is possible that the ERβ AF-1 activity dominates in some target cells and in cells in a pathophysiological state, because its activity is cell-type specific.

Several nuclear receptor coactivators have been studied, including the SRC-1/TIF2 (20–22) and p300/CREB (23–26) family proteins, TIF1 (27, 28), ARA70 (29), PGC1 (30), Smads (32), and others (31). It has been demonstrated that ligand binding induces the interactions of the SRC-1/TIF2 and p300/CREB proteins with ERα and other nuclear receptors. Most recently, a novel coactivator complex has been identified, and one of the components, TRAP220/DRIP205, is shown to bind the E/F domains of nuclear receptors in a ligand-dependent way (34, 35). Further study revealed that helix 12 at the C terminus of the E/F domains serves as a direct interface for these coactivators (38, 39). Such ligand-induced functional interactions with the coactivators will explain the ligand-induced transactivation function of AF-2 in various nuclear receptors including ERα and ERβ (40, 41). However, little was known about the coactivators for AF-1, and in this respect it is of interest whether the AF-2 coactivators enhance the AF-1 activity or not. We found in the present study that all of the three SRC-1/TIF2 family proteins and p300 potentiate the AF-2 functions of ERβ as well as ERα (data not shown), as expected from previous reports (21–23), reflecting the ligand-induced interactions of these coactivators with the E/F domains of ERα and ERβ in vitro (Fig. 3A). Under the same conditions, we could detect neither any potentiation of the AF-1 activities of ERα and ERβ by any SRC-1/TIF2 family proteins nor any direct interaction in vitro, though previous reports showed that the SRC-1/TIF2 family proteins potentiate the AF-1 activities of ERα and ERβ (2, 12, 18). As these activities are dependent on cell types, we suspect that a cell type-specific factor specifies the actions of the SRC-1/TIF2 family proteins in the ER AF-1. In contrast, p300 enhanced the AF-1 activities of both ERs, even though in the Ser residue mutants to be unphosphorylated by mitogen-activated protein kinase (data not shown). Supporting the p300 action, functional association in vivo and direct binding of p300 were detected in the A/B domains of ERα and ERβ. However, we observed a discrepancy between in vivo and in vitro in the hERβ A/B regions required for p300 binding, suggesting the existence of an unknown factor acting with p300 on the ERβ AF-1. Thus, taken together, these observations indicate that p300 is one of coactivators supporting the AF-1 activities of ERα as well as ERβ; however, unknown coactivator(s) also appears to be required for the AF-1 activities.

The present findings together indicate that p300 mediates, at least in part, the ligand-induced functional synergism between AF-1 and AF-2 through its direct binding to the A/B domains of ERα and ERβ, because direct interactions between the two domains in vitro were detected only in the presence of p300 (Fig. 5A). A previous study indicated that the SRC-1/TIF2 family proteins exhibit similar effects on the functional interaction between the two domains in vivo (18); however, in the present study, the SRC-1/TIF2 family proteins failed to induce such a ligand-induced interaction in vitro. Therefore, from the present study, it appears that such action of the SRC-1/TIF2 family proteins is mediated through p300 (possibly CBP) bound to the A/B domain. As a coactivator complex containing the SRC-1/TIF2 family proteins and p300/CBP is recruited for the ligand-induced transactivation functions of ERα and ERβ during this ligand-dependent process, p300 (CBP) in this complex may bridge the A/B and E/F domains in association of other component(s). Likewise, a component(s) of the TRAP/DRIP co-activator complex may directly interact with the ER A/B domains when this complex is recruited by the ligand-bound E/F domain (34, 35). It is more worthwhile to speculate that a coactivator complex different from these two coactivator complexes interacts with the ER A/B domain to fulfill the ER AF-1 function.

REFERENCES

1. Messman, S., Polman, J., and Dijkema, R. (1996) FEBS Lett. 392, 49–53
2. Tremblay, G. B., Tremblay, A., Copeland, N. G., Gilbert, D. J., Jenkins, N. A., Labrie, F., and Giguere, V. (1997) Mol. Endocrinol. 11, 353–365
3. Kuiper, G. G., Enmark, E., Pelto, H. M., Nilsson, S., and Gustafsson, J. A. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 5925–5930
4. Evans, R. M. (1988) Science 240, 889–895
5. Green, S., and Chambon, P. (1989) Trends Genet. 4, 309–314
6. Beato, M., Herrlich, P., and Schutz, G. (1995) Cell 83, 851–857
7. Kumar, V., Green, S., Stack, G., Berry, M., Jin, J. R., and Chambon, P. (1987) Cell 51, 941–951
8. Tora, L., White, J., Brou, C., Tasset, D., Webster, N., Scheer, E., and Chambon, P. (1989) Cell 59, 477–487
9. Berry, M., Metzger, D., and Chambon, P. (1990) EMBO J. 9, 2811–2818
10. Metzger, D., Losson, R., Bornert, J. M., Lemoine, Y., and Chambon, P. (1992) Nucleic Acids Res. 20, 2813–2817
11. Metzger, D., Ali, S., Bornert, J. M., and Chambon, P. (1995) J. Biol. Chem. 270, 9535–9542
12. Tremblay, A., Tremblay, G. B., Labrie, F., and Giguere, V. (1999) Mol. Cell. 3, 513–519
13. Kato, S., Endoh, H., Masuhage, Y., Kitamoto, T., Uchiyama, S., Sasaki, H., Masuhage, S., Gotoh, Y., Nishida, E., Kawashima, H., Metzger, D., and Chambon, P. (1995) Science 270, 1491–1494
14. Bunone, G., Briand, P. A., Mikkelsen, R. J., and Picard, D. (1996) EMBO J. 15, 2174–2183
15. Ali, S., Metzger, D., Bornert, J. M., and Chambon, P. (1992) EMBO J. 12, 1153–1160
16. Lahooti, H., White, R., Danielian, P. S., and Parker, M. G. (1994) Mol. Endocrinol. 8, 182–188
17. Le Goff, P., Montano, M. M., Schodin, D. J., and Katzenellenbogen, B. S. (1994) J. Biol. Chem. 269, 4458–4460
18. McInerney, E. M., Tsai, M. J., O’Malley, B. W., and Katzenellenbogen, B. S. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 10069–10073
19. Tasset, D., Tora, L., Fromental, C., Scheer, E., and Chambon, P. (1990) Cell 62, 117–1187
20. Cavailles, V., Dauvois, S., Danielian, P. S., and Parker, M. G. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 10009–10013
21. Onate, S. A., Tsai, S. Y., Tsai, M. J., and O’Malley, B. W. (1995) Science 270, 1354–1357
22. Voegel, J. J., Heine, M. J., Zechel, C., Chambon, P., and Gronemeyer, H. (1996) EMBO J. 15, 3667–3675
23. Kamei, Y., Xu, L., Heinzel, T., Torchia, J., Kurokawa, R., Glass, B., Lin, S. C., Heyman, R. A., Rose, D. W., Glass, C. K., and Rosenfeld, M. G. (1996) Cell 85, 403–414
24. Torchia, J., Rose, D. W., Inostroza, J., Kamei, Y., Westin, S., Glass, C. K., and Rosenfeld, M. G. (1997) Nature 387, 677–684
25. Chakravarti, D., LaMorte, V. J., Nelson, M. C., Nakajima, T., Schulman, I. G., Jugulmun, H., Montminy, M., and Evans, R. M. (1996) Nature 383, 99–103
26. Korzus, E., Torchia, J., Rose, D. W., Xu, L., Kurokawa, R., McInerney, E. M., Mullen, T. M., Glass, C. K., and Rosenfeld, M. G. (1998) Science 279, 703–707
27. LeDouarin, B., Nielsen, A. L., Garnier, J. M., Chininoise, F., Jeannoumoing, P., Losson, R., and Chambon, P. (1996) EMBO J. 15, 6701–6715
28. LeDouarin, B., Zechel, C., Garnier, J. M., Lutz, Y., Tora, L., Pierrat, P., Heery, D., Gronemeyer, H., Chambon, P., and Losson, R. (1995) EMBO J. 14, 2020–2033
29. Yeh, S., and Chang, C. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 5517–5521
30. Puigserver, P., Wu, Z., Park, C. W., Graves, R., Wright, M., and Spiegelman, B. M. (1998) Cell 94, 829–839
31. Glass, C. K., Rose, D. W., and Rosenfeld, M. G. (1997) Curr. Opin. Cell Biol. 9, 222–232
32. Yanagisawa, J., Yanagi, Y., Masuiro, Y., Suzawa, M., Watanabe, M., Kashiwagi, K., Toriyabe, T., Kawahata, M., Miyazono, K., and Kato, S. (1999) Science 283, 1317–1321
33. Chen, H., Lin, R. J., Schiltz, R. L., Chakravarti, D., Nash, A., Nagy, L., Privalsky, M. L., Nakatanai, Y., and Evans, R. M. (1997) Cell 90, 569–580
34. Fondell, J. D., Ge, H., and Roeder, R. G. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 8329–8333
35. Rasche, C., Lemon, B. D., Sildan, Z., Bromleigh, V., Gamble, M., Naar, A. M., Erdjument-Bromage, H., Tempst, P., and Freedman, L. P. (1999) Nature 398, 824–828
36. Takeyama, K., Masuiro, Y., Fuse, H., Endoh, H., Murayama, A., Kitanaka, S., Suzawa, S., Yanagisawa, J., and Kato, S. (1999) Mol. Cell. Biol. 19, 1040–1055
37. Kato, S., Sasaki, H., Suzawa, M., Masushige, S., Tora, L., Chambon, P., and Gronemeyer, H. (1995) Mol. Cell. Biol. 15, 5858–5867
38. Heery, D. M., Kalkhoven, E., Hoare, S., and Parker, M. G. (1997) Nature 387, 723–736
39. Wurtz, J. M., Bourguet, W., Renaud, J. P., Vivat, V., Chambon, P., Moras, D., and Gronemeyer, H. (1996) Nat. Struct. Biol. 3, 87–94
40. Moras, D., and Gronemeyer, H. (1996) Curr. Opin. Cell Biol. 10, 384–391
41. Freedman, L. P. (1999) Cell 97, 5–9

p300-mediated Functional Synergism in ERα and -β