CREB binding protein acts synergistically with steroid receptor coactivator-1 to enhance steroid receptor-dependent transcription

(estrogen/progesterone/p300)

CAROLYN L. SMITH*, SERGIO A. ONATE, MING-JER TSAI, AND BERT W. O’MALLEY

Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030-3498

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ABSTRACT Steroid receptors are ligand-regulated transcription factors that require coactivators for efficient activation of target gene expression. The binding protein of cAMP response element binding protein (CBP) appears to be a promiscuous coactivator for an increasing number of transcription factors and the ability of CBP to modulate estrogen receptor (ER)- and progesterone receptor (PR)-dependent transcription was therefore examined. Ectopic expression of CBP or the related coactivator, p300, enhanced ER transcriptional activity by up to 10-fold in a receptor- and DNA-dependent manner. Consistent with this, the 12S E1A adenoviral protein, which binds to and inactivates CBP, inhibited ER transcriptional activity, and exogenous CBP was able to partially overcome this effect. Furthermore, CBP was able to partially reverse the ability of active ER to squelch PR-dependent transcription, indicating that CBP is a common coactivator for both receptors and that CBP is limiting within these cells. To date, the only other coactivator able to significantly stimulate receptor-dependent transcription is steroid receptor coactivator-1 (SRC-1). Coexpression of CBP and SRC-1 stimulated ER and PR transcriptional activity in a synergistic manner and indicated that these two coactivators are not functional homologues. Taken together, these data suggest that both CBP and SRC-1 may function in a common pathway to efficiently activate target gene expression.

Steroid receptors are members of a gene superfamily of ligand-activated transcription factors that regulate many biological processes, including reproduction, differentiation, and growth. Although the biochemical events that enable hormone-bound receptor to bind to specific hormone response elements are reasonably well-defined (for reviews see refs. 1 and 2), the steps subsequent to DNA binding necessary for receptor to trans-activate target genes remain poorly understood.

It is generally thought that transcriptional activators such as steroid receptors stimulate gene expression by facilitating the assembly of basal transcription factors into a stable preinitiation complex and thereby increasing the transcription initiation rate of RNA polymerase II (3, 4). This hypothesis is supported by evidence that several steroid receptors directly interact with components of the basal transcriptional apparatus, such as the TATA box binding protein and TFIIIB (5–8).

However, efficient transcription requires additional positively acting factors termed adaptors or coactivators, which are envisioned to function as bridging factors between specific activators and general transcription factors in the preinitiation complex (9–12). Consistent with this model, recent work has demonstrated interactions between steroid receptors and the TATA box binding protein-associated factor, TAF153 (13), transcriptional intermediary factor-1 (14), thyroid hormone receptor-interacting protein-1 (15), steroid receptor coactivator-1 (SRC-1; ref. 16), and a group of receptor interacting proteins with molecular masses of 140 and 160 kDa (17–19).

Ectopic expression of these accessory factors in transient transfection assays affects gene expression in a variable manner; only SRC-1 provides a large stimulation (≥10-fold) of receptor-dependent transcription (16). However, the mechanism by which these proteins contribute to the formation of a stable preinitiation complex or to the recruitment of RNA polymerase II to target genes is unclear.

In another experimental model, it has been demonstrated recently that the coactivator of the cAMP response element binding protein (CREB) transcription factor, CREB binding protein (CBP), associates specifically with RNA polymerase II (20) as well as the phosphorylated form of CREB (21) and TFIIB (22), suggesting that this coactivator stimulates transcription, at least in part, through its recruitment of RNA polymerase II to target gene promoters. Interestingly, experimental evidence indicates that CBP also enhances the transcriptional activity of several other transcription factors (23–27) and is required for the expression of target genes regulated by promoters containing phorbol 12-myristate 13-acetate- and serum-responsive elements (26). Furthermore, during the preparation of this manuscript, Kamei et al. (28) reported that CBP physically interacts with several members of the steroid receptor superfamily and enhances retinoic acid and thyroid receptor transcriptional activity. Therefore, to determine if CBP might also contribute to estrogen receptor (ER)- and progesterone receptor (PR)-activated transcription, we undertook experiments to assess whether CBP could enhance steroid receptor-dependent transcription alone or in concert with SRC-1. Our results indicate that CBP contributes to the transcriptional activity of both of these receptors and, together with the coactivator protein, SRC-1, synergistically stimulates steroid receptor-dependent transcription.

MATERIALS AND METHODS

Plasmid DNAs. Mammalian expression vectors for human ER (pSVMT:ER) (29), the B form of human PR (pPRb) (30), the adenovirus protein 12S E1A (pCMV-12S E1A) (31), SRC-1 (pBK-CMV-SRC-1) (16), and the dominant negative form of SRC-1 [pABΔgal-SRC-1(8)] (16), as well as the ER-responsive (ERE-E1b-CAT) and PR-responsive (PRE-E1b-CAT) target gene constructs (29, 32), have been described. The UAS-E1b-LUC reporter gene contains four copies of a 17-mer upstream activating sequence upstream of the E1b TATA box and luciferase gene. The expression vectors for full-length mouse CBP and human p300, pRC/RSV-mCBP8 (21) and pRC/RSV-p300 (33), respectively, as well as pGAL-CBP, which encodes for the GAL4 DNA-binding domain fused to the N terminus of full-length mouse CBP (21).

Abbreviations: ER, estrogen receptor; PR, progesterone receptor; CREB, cAMP response element binding protein; CBP, CREB binding protein; SRC-1, steroid receptor coactivator-1; E2, 17β-estradiol; P4, progesterone; CAT, chloramphenicol acetyltransferase.

*To whom reprint requests should be addressed.

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were obtained from R. H. Goodman (Oregon Health Sciences University). To express human ER under the control of the Rous sarcoma virus promoter, a human ER cDNA was isolated from pT78b-ER (34) as a HindIII–EcoRI fragment, blunt-ended, and inserted into filled-in Asp718–BamHI sites of pRS (35). The VP16-SRC-1 chimeric expression vector was constructed by inserting the coding sequences corresponding to the VP16 activation domain (amino acids 411–487) into the BglII–BamHI sites of the pABGal mammalian expression vector (36), followed by the insertion of a blunt-ended EcoRI to SmaI fragment of the SRC-1 cDNA into the SmaI site of the same vector. The control vector used to normalize the amount of Rous sarcoma virus promoter DNA in transient transfections, pRSV-Not, was provided by R. A. Maurer (Oregon Health Sciences University).

Cell Culture Conditions and Transfections. HeLa cells were routinely maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum. Twenty-four hours before transfection, 3 × 10^5 cells were plated per well of a 6-well dish in phenol red-free DMEM containing 5% dextran-coated charcoal-stripped serum. Cells were transfected with the indicated DNAs using Lipofectin (Life Technologies, Grand Island, NY) according to the manufacturer’s guidelines. Six hours later, the DNA/Lipofectin mixture was removed and cells were washed with phenol red-free medium containing 5% stripped serum and the indicated hormones; cells were harvested 24 h thereafter. Cell extracts were assayed for chloramphenicol acetyltransferase (CAT) activity as described (29) or luciferase activity using the Luciferase Assay System (Promega). Data are presented as the average of duplicate values obtained from a representative experiment, which was independently repeated at least three times.

RESULTS

The ability of CBP to contribute to steroid receptor transcriptional activity was examined in HeLa cells expressing the human ER. Under our experimental conditions, ectopic expression of mouse CBP increased 17β-estradiol (E2)-stimulated ERE-E1b-CAT gene expression by 10.0 ± 0.9-fold (mean ± SEM, n = 5) when compared with hormone-stimulated activity in the absence of exogenous CBP (Fig. 1A). Exogenous CBP expression also enhanced the transcriptional activity of ER in the absence of exogenous ligand, but to a lesser extent (6.0 ± 1.3-fold; Fig. 1A). To ascertain that the effect of CBP on transcription depended on ER bound to its hormone response element, CAT gene expression was determined for cells lacking ER or cells transfected with a PRE-E1b-CAT target gene, which contained two progesterone (P4) response elements to which ER cannot bind (37). In neither instance was there any significant increase in CAT activity observed. Thus, the ability of CBP to enhance target gene expression required the presence of receptor and its cognate DNA binding site.

CBP and E1A-associated p300 are closely related proteins that exhibit >85% identity within the regions proposed to comprise their common functional domains (38). Furthermore, CBP and p300 appear to be functional homologues of one another with respect to their roles as coactivators of CREB (33, 39). We therefore asked whether p300 could also modulate ER-dependent transcription and found that when similar amounts of the p300 expression vector were transfected into cells, gene expression was stimulated 6.1 ± 0.6-fold (n = 3; Fig. 1B). Thus CBP and p300 both positively modulate ER transcriptional activity.

The adenovirus E1A gene product binds to the third zinc-finger motif of CBP and inactivates its function as a coactivator of CREB-mediated transcription (33). Therefore, to test whether endogenous E1A binding proteins such as CBP are necessary for steroid receptor transactivation in cells, the effect of 12S E1A expression on ER-dependent transcription was examined. Interestingly, 12S E1A blocked ER-dependent transcription in a dose-dependent manner (Fig. 2A) and when >0.5 μg of 12S E1A expression vector was transfected into these cells, ER transcriptional activity was completely abrogated (data not shown). Since adenovirus E1A gene products bind to multiple cellular proteins, including p300 and so-called “pocket” proteins such as the retinoblastoma gene product, Rb, (40, 41), it was possible that 12S E1A blocked ER transcriptional activity via inhibition of other factors required for gene expression. Therefore to determine if CBP was an E1A target critical for ER-stimulated transcription, CAT activity was measured in cells transfected with an amount of 12S E1A plasmid sufficient to block ~80% of ER transcriptional activity and increasing levels of CBP expression vector. Ectopic CBP increased target gene expression inhibited by E1A in a dose-dependent fashion (Fig. 2B), and this supports the hypothesis that CBP in its role as a putative coactivator is at least one of the molecular coactivator components critical for efficient ER transactivator of target gene expression.

The ability of activated ER to inhibit PR-dependent gene expression has been interpreted to suggest that these transcription factors use a common pool of coactivators that are present in limiting cellular concentrations (42, 43). To assess whether CBP could be at least one of the coactivators sequestered by ER in these experiments, the ability of CBP to reverse the squelching of PR transcriptional activity by E2-stimulated ER was examined. As shown in Fig. 3, P4-stimulated expression of the PRE target gene was reduced ~95% by estrogen-activated ER and, in a dose-dependent manner, exogenous

![Fig. 1. Ectopic expression of CBP and p300 enhances ER-dependent transcription. (A) HeLa cells were transfected with (+) or without (−) 0.5 μg of pRSV-ER (ER) in the presence (+) or absence (−) of 5 μg of pRc/RSV mouse CBP and treated with 1 nM E2 (+) or ethanol vehicle (−). Activation of target gene expression containing (ERE-E1b-CAT) or lacking (PRE-E1b-CAT) an estrogen response element was measured. (B) Cells transfected with 0.5 μg of pRSV-ER and 2.5 μg of pRSV-ERE1b-CAT in the presence (+) or absence (−) of 5 μg of pRc/RSV p300 expression vector were treated with 1 nM E2 (+) or vehicle (−).]
Fig. 2. Exogenous expression of CBP stimulates ER transcriptional activity inhibited by 12S E1A. (A) HeLa cells were transfected with 0.5 μg of pRc/RSV-ER and 2.5 μg of ERE-E1b-CAT (ER) in the absence (−) or presence of increasing amounts (given in μg) of pCMV-12S E1A. (B) Cells were transfected with 0.5 μg of pRc/RSV-ER and 2.5 μg of ERE-E1b-CAT in the absence (−) or presence (+) of 0.1 μg E1A expression vector and increasing amounts (given in μg) of pRc/RSV-CBP. In both A and B, cells were treated with 1 nM E2 (+) or ethanol (−) and E2-stimulated CAT activity in the absence of exogenous CBP and/or E1A was defined as 100.

CBP increased this level of CAT activity by 27-fold. The restoration of transcriptional activity is not consistent solely with an enhancement of the residual active PR, since ectopic CBP expression in the absence of activated ER increased PR transcriptional activity to a lesser extent (13-fold). Thus, CBP functions as a common coactivator for both ER and PR and, under these experimental conditions, cellular CBP concentrations appear to be limiting.

Since the only other ER- or PR-interacting coactivator protein described to date that exhibits significant transcription enhancing activity is SRC-1 (16), the ability of CBP and SRC-1 to alter gene expression individually and in combination with each other was examined to determine if they were functionally equivalent (Fig. 4). In the experiment shown in Fig. 4A, both SRC-1 and CBP stimulated ER transcriptional activity to an equivalent extent (6- to 7-fold). However, when they were provided simultaneously to the cell, the increase in gene expression was synergistic (~38-fold). Similarly, PR transcriptional activity in cells transfected with CBP and SRC-1 expression vectors was more than additive relative to that observed in the presence of CBP or SRC-1 alone (Fig. 4B).

Although several mechanisms for the synergistic activation of ER and PR transcriptional activity by SRC-1 and CBP could be envisioned, the simplest model would rely upon direct interaction between these two proteins. Consistent with this hypothesis, we have observed that CBP interacts with SRC-1 in vivo as assessed with a mammalian two-hybrid system using a "bait" protein, consisting of the GAL4 DNA-binding domain linked to full-length CBP and the VP-16 activation domain fused to full-length SRC-1 (data not shown).

Fig. 3. Exogenous expression of CBP reverses squelching of PR transcription by ER. Cells were transfected with 1 μg each of pSVMT:wER and phPRB (which use identical promoter and enhancer to regulate expression), 2 μg of PRE-E1b-CAT and increasing amounts (given in μg) of CBP expression vector and subsequently treated with ethanol vehicle (−) or 1 nM E2 or 10 nM P4.

Fig. 4. Simultaneous ectopic expression of CBP and SRC-1 enhances ER and PR transcriptional activity synergistically. (A) Cells were transfected with 0.5 μg of pRc/RSV-ER, 2.5 μg of ERE-E1b-CAT, and 1 μg of pCMV-SRC-1 and/or 5 μg of pRc/RSV-CBP and treated with 1 nM E2. (B) Cells were transfected with 1 μg of phPRB, 2.5 μg of PRE-E1b-CAT, and 0.5 μg of pCMV-SRC-1 and/or 2.5 μg of pRc/RSV-CBP and subsequently exposed to 10 nM P4. In both A and B, transcriptional activity in the presence of the appropriate hormone, but absence of exogenous SRC-1 or CBP, was defined as 1. Cells in which SRC-1 or CBP expression vectors were not introduced were transfected instead with pBK-CMV (Stratagene) or pRSV-Not, respectively.
DISCUSSION

Ectopic expression of either CBP or p300 markedly increased E2- and P2-stimulated ER and PR gene expression and suggests that these proteins significantly contribute to the ability of these steroid receptors to trans-activate target genes. Although p300 appeared to stimulate gene expression to a lesser extent than CBP in these experiments, it is not possible to conclude that CBP is more physiologically relevant than p300 without determining the relative endogenous and exogenous expression of both coactivators in a given target cell. Gene expression was not enhanced by ectopic CBP in the absence of steroid receptor nor did CBP stimulate CAT activity in the absence of the appropriate DNA sequence for receptor binding. These results indicate that CBP was not simply increasing the activity of factors able to bind to the minimal promoter element and suggests that estrogen response element-bound, activated ER may recruit CBP to the pre-initiation complex.

The ability of E1A to block ER transcriptional activity suggests that E1A interacts with and inhibits proteins required for receptor-dependent transcription. This is unlikely to be the result of a decreased ER within the cell, since E1A does not inhibit expression from the Rous sarcoma virus long terminal repeat (44) used to direct ER expression in these experiments. Therefore, the ability of exogenous CBP to increase gene expression by 3-fold in the presence of E1A is consistent with at least a portion of the E1A inhibition the result of its interaction with CBP. However, in addition to CBP and p300, 12S E1A does bind to several other proteins, such as the transcriptional repressor Dr1 (45), the YY1 transcription factor (46), and TFID/TATA box binding protein (47), and, without quantitation of CBP and E1A levels in target cells, the possibility remains that a fraction of the lost ER transcriptional activity may be the result of E1A sequestration of a molecular target other than CBP. Although one such potential target could be SRC-1, our preliminary data suggests that E1A does not alter the intrinsic transcriptional activity of a GAL4–SRC-1 fusion protein.

Squelching experiments in which one activator inhibits the target gene trans-activation by another activator protein suggested that coactivators are necessary for efficient transcription and that they are present in limiting concentrations within cells (10). We have demonstrated that exogenous SRC-1 reverses the squelching of PR transcriptional activity by E2-activated ER, indicating that SRC-1 was necessary for efficient ER and PR transactivation (16). Since SRC-1 binds to the ligand binding domain of PR, the ability of the ER A/B domain alone to inhibit PR transcriptional activity (42) suggests that additional factors necessary for optimal receptor dependent transcription may also be required. The ability of exogenous CBP to enhance P2- and E2-stimulated gene expression and to reverse ER squelching of PR transcription supports the hypothesis that CBP is one of the additional coactivators for both ER- and PR-dependent transcription and is limiting in the HeLa cells used in our experimental model. Although we favor the hypothesis that SRC-1 and CBP act synergistically, each may be capable of functioning alone, since we observe partial reversal of squelching by a single coactivator in receptor–receptor squelching experiments. Even if SRC-1 and CBP function together, partial reversal might be expected at high concentrations of either molecule, since overexpression would enhance weak protein–protein interactions at both the parent (receptor) complex and the downstream target.

The ability of exogenous CBP and SRC-1 to synergistically enhance ER and PR transcriptional activity indicates that these two coactivators are not functionally equivalent but likely act via a common pathway. This synergistic effect might be achieved through enhanced stability of steroid receptor-dependent, protein–protein interactions in the presence of elevated levels of CBP and SRC-1. In agreement with our finding of a functional interaction between SRC-1 and CBP in intact cells, Kamei et al. (28) have demonstrated that a subregion of the glutamine-rich carboxy terminus binds to a high molecular weight variant (p160) of SRC-1 in vitro. This, taken together with the ability of the amino terminus of CBP and the carboxy terminus of SRC-1 to interact with the ligand binding domain of several steroid receptors, suggests that a ternary complex of CBP, liganded nuclear receptor and SRC-1/p160 may be present at target gene promoters (16, 19, 28). Alternatively, CBP and SRC-1 may function synergistically via interaction and/or recruitment of distinct proteins (e.g., general transcription factors) necessary for optimal transcription. Both RNA polymerase II and TFIIIB bind to CBP (20, 22), and it will be of particular interest to determine if SRC-1 interacts with these or other components of the general transcriptional machinery.

The CBP/p300 coactivators are known to bind to and/or stimulate the activity of an increasing number of transcription factors including CREB (22, 26), MyoD (48), c-Myb (23), c-Jun (27), c-Fos (24), and E2F1 (25). While it is not known whether the presence of CBP is required for SRC-1 functional activity, CBP does serve as a coactivator for several transcription factors (CREB, E2F) whose activities are not influenced by exogenous SRC-1 expression (16), indicating that the ability of CBP to enhance gene expression is more general and not strictly dependent upon SRC-1.

Kamei et al. (28) have proposed that CBP be termed an “integrator” for diverse signals converging at nuclear target genes. The ability of CBP to serve as a coactivator/integrator of a broad array of transcription factors raises interesting questions regarding the role of CBP/p300 in cells in which multiple transcription factors, including nuclear receptors, may be simultaneously active. For instance, does CBP/p300 have the same affinity for all activators, or is this coactivator preferentially recruited by specific transcription factors at the expense of others resulting in differential expression of CBP/p300 target genes? In addition, the ability of CBP to integrate transcription initiated by protein kinase A and growth factor signal transduction pathways raises the question as to whether CBP may play a role in the activation of ligand-independent ER transcriptional activity stimulated by cAMP and epidermal growth factor.

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