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P/CAF associates with cyclin D1 and potentiates its activation of the estrogen receptor

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ABSTRACT **Cyclin D1 is overexpressed in a significant percentage of human breast cancers, particularly in those that also express the estrogen receptor (ER). We and others have demonstrated previously that experimentally overexpressed cyclin D1 can associate with the ER and stimulate its transcriptional functions in the absence of estrogen. This effect is separable from the established function of cyclin D1 as a regulator of cyclin-dependent kinases. Here, we demonstrate that cyclin D1 can also interact with the histone acetyltransferase, p300/CREB-binding protein-associated protein (P/CAF), thereby facilitating an association between P/CAF and the ER. Ectopic expression of P/CAF potentiates cyclin D1-stimulated ER activity in a dose-dependent manner. This effect is largely dependent on the acetyltransferase activity of P/CAF. These results suggest that cyclin D1 may trigger the activation of the ER through the recruitment of P/CAF, by providing histone acetyltransferase activity and, potentially, links to additional P/CAF-associated transcriptional coactivators.**

The D type cyclins have a well established role in regulating progression through G_1 phase of the cell cycle. In response to extracellular mitogenic signals, they are synthesized and assembled together with cyclin-dependent kinase CDK4 or CDK6 into holoenzymes that phosphorylate the retinoblastoma susceptibility gene product, RB. In the context of cell cycle regulation, RB functions as a transcriptional repressor protein that, in its underphosphorylated state, inhibits entry into S phase of the cell cycle by binding and inactivating the E2F transcription factor. Free E2F stimulates the synthesis of enzymes that are required for DNA replication. By initiating the phosphorylation of RB, cyclin D-dependent kinases inactivate its ability to restrain progression through G_1 into S phase (1). Consistent with the physiological role of the D type cyclins in promoting cell proliferation, cyclin D1 is an oncogene and has been found to be amplified or overexpressed in a variety of tumors (2).

Results from several groups suggest that D type cyclins also may intervene in the activities of transcription factors through CDK-independent mechanisms. All three D type cyclins can bind to and repress the activity of a recently characterized myb-like transcription factor, DMP1, in a CDK-independent manner (3, 4). Cyclins D1 and D2 have been reported to inhibit transcriptional activation by v-Myb (5). Cyclin D1, but not cyclin D3, is able to inhibit myoblast differentiation by repressing the activity of the myogenic transcription factor MyoD in what may be a CDK-independent manner (6, 7). Although all three D type cyclins are able to collaborate with CDK4 and CDK6 to accomplish RB phosphorylation, they exhibit tissue-specific differences in their expression patterns. It is possible that CDK-independent transcriptional effects may contribute to tissue-specific functions of D type cyclins.

Deregulation of cyclin D1 expression has dramatic effects in the breast. Inactivation of the cyclin D1 gene in mice severely impairs mammary gland development during pregnancy (8, 9). Targeted overexpression of cyclin D1 in the mammary glands of transgenic mice led to the development of mammary hyperplasia and adenocarcinomas (10). More than 50% of human breast cancers have been found to overexpress cyclin D1. Among these, up to 20% involve an amplification of the gene, suggesting that cyclin D1 overexpression may play a causal role in carcinogenesis (11). Overexpression of cyclin D1 in breast tumors correlates with the expression of the estrogen receptor (ER) (12, 13).

The ER belongs to a family of structurally conserved transcriptional activators, the nuclear receptors, which stimulate the expression of specific genes in the presence of their respective ligands (14). The ER is required for cell proliferation or differentiation in many estrogen-dependent tissues, including the breast (15).

Our laboratory recently has demonstrated that, in a mouse mammary epithelial cell line (SCp2), which can be induced to differentiate into milk-producing, gland-like structures, cyclin D1 expression increased over the course of differentiation. The rise in cyclin D1 levels coincided with a decrease in CDK4 protein expression and associated kinase activity and with induction of the transcriptional activity of the ER, although the experiment was conducted in estrogen-free medium (16). Consistent with these observations, we found that ectopically expressed cyclin D1 could associate physically with the ER and stimulate its transcriptional functions through a CDK-independent mechanism (16). Similar conclusions were reached independently by others (17). Because ER-expressing breast tumors often occur in postmenopausal women—who have low levels of circulating estrogens—it is possible that the frequently overexpressed cyclin D1 may, at least in part, be responsible for stimulating ER activity in this setting. Therefore, we are interested in elucidating the mechanism by which cyclin D1 can function as a ligand for the ER.

Estrogens activate the ER by inducing a conformational change in its hormone-binding domain (18, 19), thereby creating a surface that binds to a number of transcriptional coactivators, such as SRC-1/N-CoA1 and AIB1/RAC3/ACTR/P/CIP (20–26). These coactivators are thought to recruit additional transcriptional cofactors, P/CAF and p300/CBP (27–29), which also make independent contacts with the nuclear receptor, as well as with one another (21, 30). P/CAF and p300/CBP promote transcription by acetylating histones and thereby altering the chromatin structure (31–33).

Abbreviations: ER, estrogen receptor; CBP, CREB-binding protein; P/CAF, P300/CBP-associated protein; CDK, cyclin-dependent kinase; SRC-1, steroid receptor coactivator 1; AIB1, amplified in breast cancer 1; HA, hemagglutinin antigen; HAT, histone acetyltransferase; RB, retinoblastoma susceptibility gene product.

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Activation of ER-mediated transcription by cyclin D1 presumably requires changes in the chromatin structure similar to those effected by the hormone-bound ER, suggesting that cyclin D1 may promote an association between the ER and one or more coactivators. Here we report that cyclin D1 has the ability to associate with P/CAF, facilitating the formation of a ternary complex in which P/CAF associates with the ER. P/CAF potentiates the transcriptional activation of the ER in a cyclin D1-dependent manner.

MATERIALS AND METHODS

Cells and Cell Culture. SAOS-2 and COS cells were maintained in DMEM (GIBCO) containing 10% FBS (HyClone), penicillin, and streptomycin. MCF-7 cells were grown in DMEM supplemented with 10% FBS and 5 μg/ml of insulin, penicillin, and streptomycin and treated with 25 nM phorbol 12-myristate 13-actetate (Sigma) after transfection.

Plasmids. An estrogen-responsive luciferase reporter construct, pGL3-$(ERE)_2$TATA-luc, was made by using the vector pGL3-basic (Promega). A double-stranded oligonucleotide (sense strand: 5′-CAGGTCACTGTGACCTATCGATAGGTCACTGTGACC-3′; antisense strand: 5′-TCGAGGTCACAGTGACCTATCGATAGGTCACAGTGACCTGGTAC-3′), containing two copies of the estrogen-response element found in the vitillogenin A promoter as well as a *Kpn*I site and a *Xho*I site (underlined), was ligated together with a 50-bp *Sal*I-*Bam*HI fragment containing the TATA box from the EIB promoter into pGL3 basic that had been digested with *Kpn*I and *Bgl*I. The plasmids pRcCMV-cyclin D1, pRcCMV-cyclin D1-HA, pRcCMV-cyclin D2-HA, pRcCMV-cyclin D3-HA, and pCX-FLAG-P/CAF have been described (7, 16, 33). To express cyclin D1 fused at the amino terminus to the Gal4 DNA-binding domain, an in-frame *Eco*RI site was introduced immediately upstream of the start codon of cyclin D1 by site-directed mutagenesis using the Mutagene kit (Bio-Rad). The cyclin D1 coding sequence then was transferred into pCMX-Gal4 (34). A FLAG epitope-tagged ER was generated by amplifying the coding sequence of the human ER by PCR using the primers 5′-CGTACCTCGAGGCCACCATGGACTACAAGGACGACGATGACAAAATCGATATGACCATGACCCTCCAC-3′ and 5′-GCGCATTTGCGGCCGCGGCGTTGAACTCGTA-3′. This resulted in a fragment with an *Xho*I site (underlined), a Kozak consensus sequence, a sequence encoding the FLAG epitope (MDYKDDDK), followed by a *Cla*I (underlined) site and the beginning of the human ER coding region up to a natural *Not*I (underlined) site. This fragment then was digested with *Xho*I and *Not*I and ligated into the plasmid pcDNA3.1-hER, which likewise had been cut with *Xho*I and *Not*I. A derivative of P/CAF with a double-point mutation in the acetyl-CoA-binding domain, hereafter referred to as HAT(−) (histone acetyltransferase), was constructed by mutating the tyrosine at position 616 and phenylalanine at 617 to alanines by site-directed mutagenesis (30, 35).

Transfection, Immunoprecipitation, and Western Blotting. For transfection studies, COS cells were plated in 60-mm dishes and transfected by the DEAE-dextran method with the indicated combinations of the plasmids to a total of 6 μg DNA per plate. SAOS-2 cells were split into 100-mm plates and transfected by the calcium phosphate method with a total of 30 μg DNA per plate. MCF-7 cells were transfected with 6 μg DNA per 100-mm plate by using Fugene-6 (Boehringer Mannheim). Forty-eight hours later, the cells were lysed in EBC buffer (50 mM Tris·HCl, pH 8/120 mM NaCl/0.5% Nonidet P-40) containing the protease inhibitors leupeptin (2 μg/ml), aprotinin (2 μg/ml), and PMSF (100 μg/ml). Immunoprecipitations were carried out with the M2 antibody against the FLAG epitope (Sigma), an antibody against the Gal4 DNA-binding domain (RK5CI, Santa Cruz Biotechnology), or an antibody against the ER (AER 314; NeoMarkers) by using a rabbit anti-mouse secondary antibody (Sigma) and protein A-Sepharose beads (Pharmacia). Precipitated proteins were resolved by SDS/PAGE, transferred to Immobilon membranes (Millipore), and probed with the M2 anti-FLAG antibody, a rabbit polyclonal antibody against cyclin D1 (Ab3, NeoMarkers, Union City, CA), the 12CA5 antibody against the hemagglutinin antigen-epitope (HA), or a mAb against the ER (TE111, NeoMarkers), as indicated in the figure legends. Reactive bands were visualized by using horseradish peroxidase-conjugated secondary antibodies and enhanced chemiluminescence (Amersham). For metabolic-labeling experiments, cells were transfected as indicated and, 48 hr later, metabolically labeled with [^{35}S]methionine (New England Nuclear) for 4 hr.

Transcriptional Activation Assays. Twenty-four hours before transfection, SAOS-2 cells were split into six-well dishes at a density of 1.8×10^5 cells per well in phenol-red-free DMEM (GIBCO) supplemented with 10% charcoal-dextran-stripped FBS (HyClone). Cells then were transfected by the calcium phosphate method with the indicated plasmids. Precipitates were washed off the cells 18–20 hr after transfection. Forty-eight hours posttransfection, the cells were lysed in a Triton buffer (25 mM Tris·PO_4, pH 7.8/2 mM 1,2-diaminocyclohexane-*N*,*N*,*N*′,*N*′-tetraacetic acid/10% glycerol/1% Triton X-100/2 mM DTT) and luciferase activity was measured (36). β-Galactosidase activity also was determined as an internal control for transfection efficiency.

Assay for Histone Acetyltransferase Activity. Plasmids encoding FLAG-P/CAF or FLAG-P/CAF-HAT(−) were transfected into COS cells by using the DEAE-dextran method. Cells were lysed in EBC buffer supplemented with butyric acid (10 mM) and subjected to immunoprecipitation with the M2 anti-FLAG antibody. Precipitated proteins were incubated for 30 min at 30°C in 30 μl HAT buffer (100 mM Tris·HCl, pH 8/20% glycerol/2 mM DTT/2 mM PMSF/0.2 mM EDTA/0.1 M NaCl/0.02 M butyric acid) with 20 μg calf thymus histones (Boehringer Mannheim) and 0.12 μCi ^{14}C-acetyl CoA (New England Nuclear) (32).

RESULTS

P/CAF Associates with Cyclin D1. To assess the ability of cyclin D1 to associate with P/CAF, COS cells were transfected with expression plasmids encoding FLAG-epitope tagged P/CAF and cyclin D1. Immunoblotting revealed that cyclin D1 could be precipitated with an antibody against the FLAG epitope in the presence, but not in the absence, of FLAG-P/CAF (Fig. 1*A*). Reciprocally, FLAG-P/CAF could be coprecipitated with a Gal4-cyclin D1 fusion protein, but not with Gal4 alone, by using an antibody against Gal4 (Fig. 1*A*). Thus, cyclin D1 and P/CAF can associate when they are cotransfected into COS cells, which allow for relatively high levels of overexpression. Comparing the amounts of cyclin D1 or Gal4-cyclin D1 and FLAG-P/CAF present in the COS cell lysates with the amounts that were precipitated in each immune complex, we estimated that approximately 2% of the cyclin D1 expressed in COS cells could be found in a complex with P/CAF. We were not able to detect an association between *in vitro* translated and bacterially produced cyclin D1 and P/CAF proteins, which raises the possibility that the association *in vivo* might be mediated by another protein or a posttranslational modification of one or both proteins. However, in an immunoprecipitation of FLAG-P/CAF from SAOS-2 cells that have been radiolabeled for 4 hr, we did not detect any P/CAF-dependent coprecipitating bands aside from cyclin D1 (Fig. 1*B*). The amount of cyclin D1 that could be precipitated with FLAG-P/CAF is comparable to that which could be coprecipitated with FLAG-ER (Fig. 1*B*).

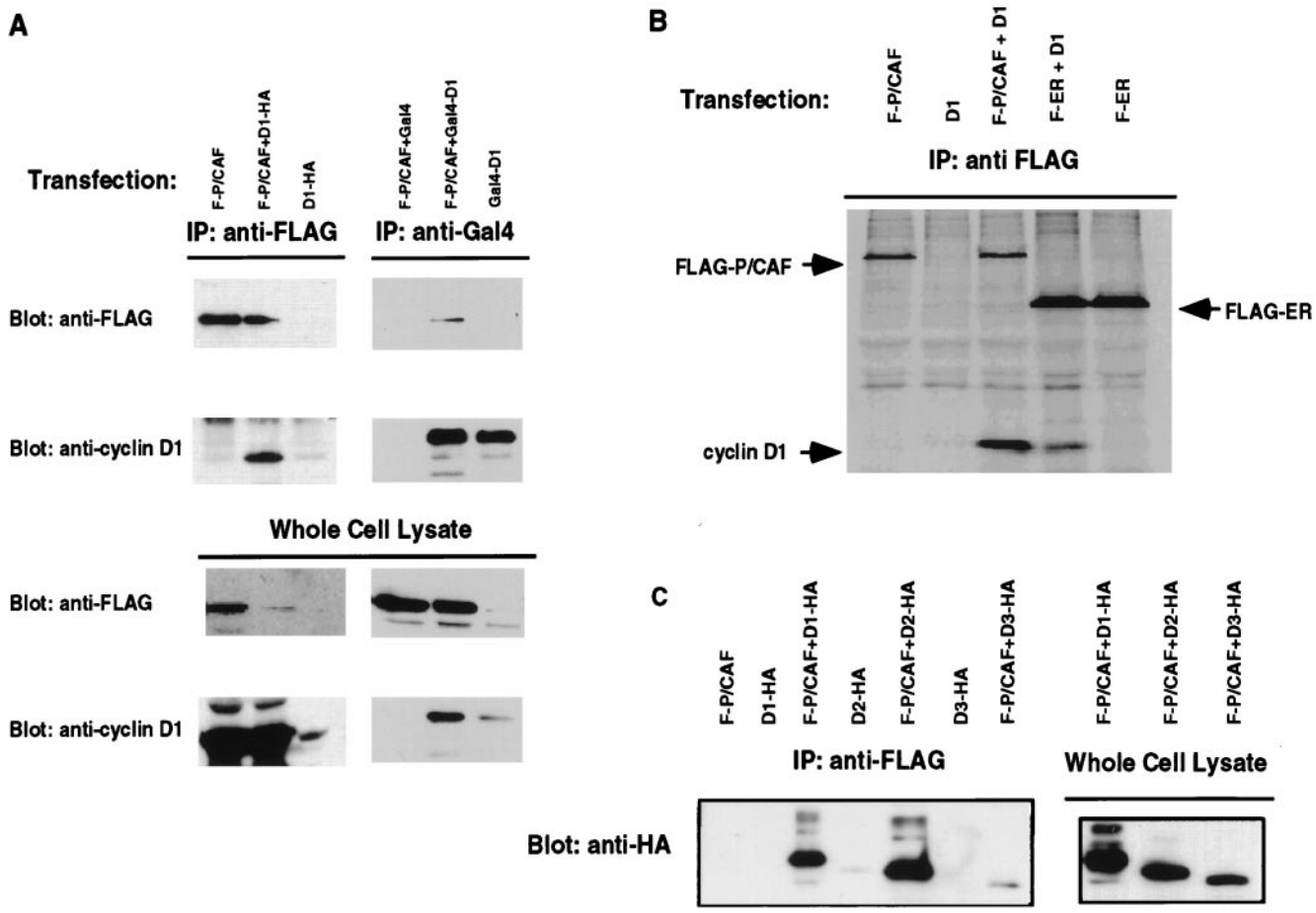

FIG. 1. The association between P/CAF and cyclin D1 *in vivo*. (*A*) COS cells were transfected with the indicated combinations of pCX-FLAG-P/CAF (F-P/CAF) and pRcCMV-cyclin D1-HA, pCMX-Gal4-cyclin D1, and pCMX-Gal4. Immunoprecipitations were carried out with antibodies against the FLAG epitope or the Gal4 DNA-binding domain. Precipitated proteins were separated by SDS/PAGE on a 10% gel, transferred to a polyvinylidene difluoride membrane, and analyzed by Western blotting with antibodies against cyclin D1 or the FLAG epitope. (*Lower*) Whole-cell lysate equivalent to one-tenth of the input for each immunoprecipitation. (*B*) SAOS-2 cells were transfected with the indicated combinations of FLAG-P/CAF, FLAG-ER, and cyclin D1 expression plasmids. They then were metabolically labeled with [^{35}S]methionine for 4 hr, and immunoprecipitations were carried out with the M2 antibody. Precipitated proteins were resolved on a 10% gel and detected by autoradiography. (*C*) COS cells were transfected with pRcCMV-cyclin D1-HA, pRcCMV-cyclin D2-HA, or pRcCMV-cyclin D3-HA, plus either pCX-FLAG-P/CAF or the empty vector. Immunoprecipitations were carried out with anti-FLAG antibodies. Proteins were detected by Western blotting with an antibody against the HA-epitope.

We have reported previously that cyclin D2 and cyclin D3 also can bind to the ER and activate its transcriptional functions, although not as effectively as cyclin D1 (15). Therefore, we assessed the ability of P/CAF to bind to other D type cyclins. COS cells were transfected with plasmids encoding FLAG-P/CAF and cyclin D1-HA, cyclin D2-HA, or cyclin D3-HA. Anti-FLAG immunoprecipitates were resolved on a denaturing gel and analyzed by Western blotting with an anti-HA antibody, revealing that cyclin D2 also could be readily coprecipitated with P/CAF. Cyclin D3-HA was expressed less abundantly than cyclins D2 and D1, but could still associate with P/CAF, although to a much lesser extent (Fig. 1*C*). This is consistent with our previous observation that each of the D type cyclins is able to induce some degree of ER activation.

Because cyclin D1 binds to both P/CAF and the ER *in vivo*, we asked whether or not cyclin D1 might mediate the assembly of a complex containing both P/CAF and the ER. We were able to detect a ternary complex involving cyclin D1, P/CAF, and the ER most readily in the human breast cancer cell line MCF-7, induced to differentiate with phorbol esters. The ER coprecipitated with FLAG-P/CAF in the presence, but not in the absence, of cotransfected cyclin D1 (Fig. 2). The cotransfection of cyclin D1 did not increase the expression of the ER. Therefore, the cyclin D1-dependent coprecipitation of the ER with P/CAF suggests that cyclin D1 creates or stabilizes a complex between P/CAF and the ER through its interactions with each protein.

P/CAF Cooperates with Cyclin D1 to Activate the ER. The observation that cyclin D1 can mediate an interaction between the ER and P/CAF led us to investigate the possibility that P/CAF and cyclin D1 might cooperate in stimulating the transcriptional functions of the ER. We used SAOS-2 cells for these experiments because they do not express detectable levels of cyclin D1. The cells were transfected with an estrogen-responsive luciferase reporter construct, plasmids encoding the ER, either cyclin D1 or the empty vector, and increasing amounts of a P/CAF expression vector. We found that cotransfecting P/CAF led to a dose-dependent increase in cyclin D1-stimulated ER activity. Neither P/CAF, cyclin D1, nor the combination stimulated the reporter in the absence of the ER, supporting the notion that these proteins must assemble together with the ER on the promoter to exert their effects on transcription (Fig. 3*A*). P/CAF did not affect the basal activity of the ER, nor did it consistently potentiate estradiol-induced activity.

P/CAF promotes transcription through its ability to acetylate histones. To determine whether or not this catalytic

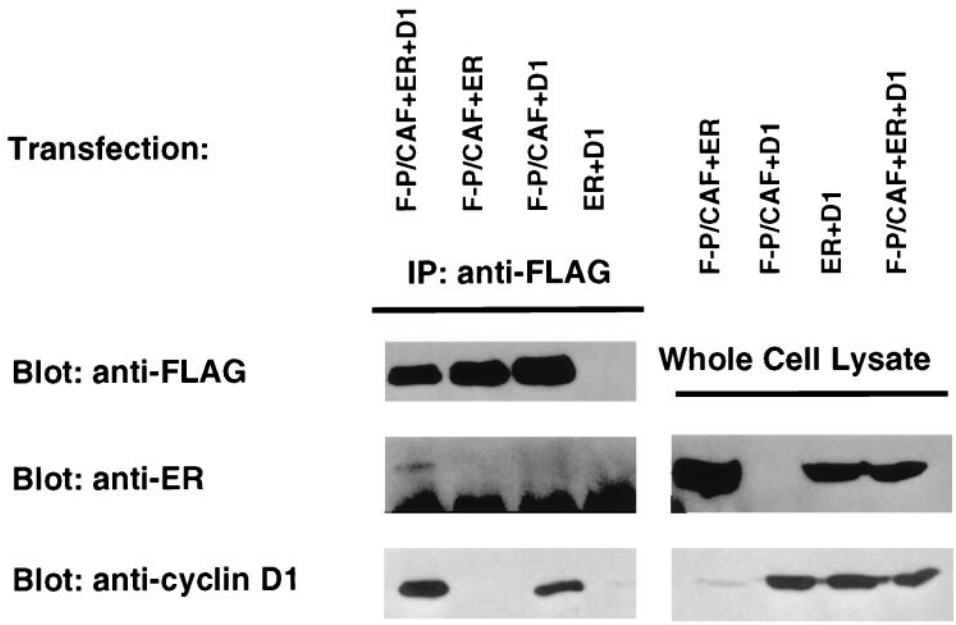

FIG. 2. Cyclin D1-dependent recruitment of the ER into a complex with P/CAF. MCF-7 cells were transfected with the indicated combinations of pCX-FLAG-P/CAF (2 μg), pcDNA3.1-hER (1 μg), pRcCMV-cyclin D1 (2 μg), plus the empty pRcCMV vector to make up a total of 6 μg DNA. Immunoprecipitations were carried out with the anti-FLAG antibody. Precipitated proteins were analyzed by SDS/PAGE followed by Western blotting of the indicated antibodies.

function is required for the effects of P/CAF on cyclin D1-stimulated ER activity, we mutated residues known to be critical for histone acetyltransferase (HAT) activity (30, 35). The resulting mutant was unable to acetylate histones or to autoacetylate itself in an *in vitro* acetylation assay (Fig. 3*B*). Although retaining the ability to bind to cyclin D1 as efficiently as the wild type in coimmunoprecipitation experiments (Fig. 3*C*), the HAT-deficient mutant was severely deficient in potentiation of cyclin D1-mediated ER activity (Fig. 3*A*).

DISCUSSION

Our data demonstrate that P/CAF can associate with cyclin D *in vivo* and can be recruited into a complex with the ER by cyclin D1. There have been several reports that P/CAF associates with nuclear receptors, including the ER, in a ligand-independent manner *in vitro* and in yeast. However, in mammalian cells, P/CAF has been shown to interact only with liganded nuclear receptors (30, 37). Under the conditions used in our experiments, no interaction was observed between the estrogen receptor and P/CAF except when cyclin D1 was overexpressed. Furthermore, we found that coexpression of P/CAF specifically enhances the activation of the ER by cyclin D1. These data are consistent with a model in which cyclin D1 recruits P/CAF to the ER and thereby promotes the formation of a transcriptionally active complex.

P/CAF, as well as p300/CBP, SRC-1, and AIB1, have been shown to acetylate nucleosomal histones *in vitro* (31–33). The acetylation of histones is thought to facilitate transcription by altering the chromatin structure to render it more accessible to the transcription machinery. P/CAF and p300/CBP have also been shown to acetylate the transcription factors TFIIEb and TFIIF (38), and the transcriptionally active tumor-suppresser protein p53 has been shown to undergo functionally relevant acetylation by p300 and P/CAF (39, 40). P/CAF has been shown to potentiate the transcriptional activity of MyoD through a mechanism that requires an intact HAT domain (41). Because the potentiation of cyclin D1-mediated ER activity is reduced sharply when the acetyltransferase function of P/CAF is disrupted, it seems likely that the effect of P/CAF on cyclin D1-stimulated ER activity also involves acetylation of histones or other substrates. However, we did not find the HAT(−) mutant to exhibit a dominant-negative effect. In fact, the HAT(−) mutant still retains the ability to increase the cyclin D1-ER activity to a very slight degree. This residual effect suggests that P/CAF may promote transcription through additional mechanisms or that HAT activity can be provided by other P/CAF-associated proteins.

P/CAF originally was identified based on its homology to the yeast HAT enzyme GCN5, a factor that is necessary for the function of certain promoter-specific transcriptional activators containing acidic activation domains (33). In yeast, GCN5 is one component of a large complex of proteins that participate in various aspects of transcription, known as the SAGA complex (42). This contains two HAT-helper proteins, Ada2 and Ada3, which are necessary for the acetylation of histones by GCN5. The SAGA complex also contains Spt proteins, which affect transcription through interactions with the TATA-binding protein, TBP, as well as several TBP-associated factors or TAFs (43). By interacting with TBP and the acidic activating domains of gene-specific transcriptional activators, the SAGA complex not only provides HAT activity, but also serves to couple the general transcriptional machinery to specific activator-responsive promoters. P/CAF and its close relative, human GCN5, also have been found in complexes that are similar to the yeast SAGA complexes in that they contain human homologues of the yeast Ada2 and Ada3, as well as several SAGA-associated TAFs (44). If the P/CAF-containing complex is functionally analogous to the SAGA complex, then it may be expected to facilitate transcription by supplying a link to the basal transcription machinery, as well as HAT activity, and a link to activators (such as the ER). Consistent with this, both P/CAF and its associated protein, p300/CBP, have been found in complexes that contain RNA polymerase II (45, 46). The potential role of the SAGA-like P/CAF complex in ER activation is suggested further by the finding that Ada3, a component of both SAGA and P/CAF complexes, associates with the ER and potentiates its activity in yeast and mammalian cells (47). Another component of the P/CAF complex, TAFII30, was identified originally based on its ability to constitutively bind to the ER in a ligand-independent manner and to be required for transcriptional activity *in vitro* (48).

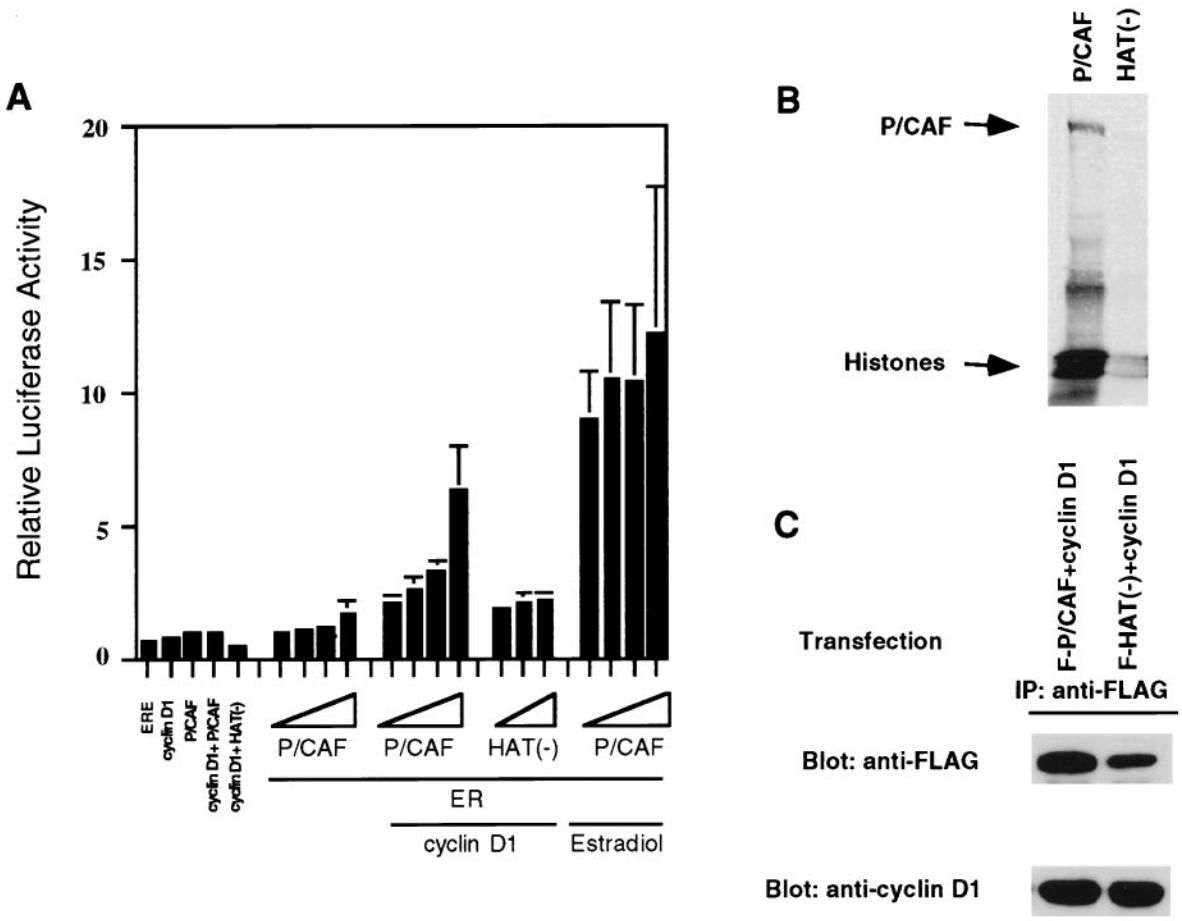

FIG. 3. Potentiation of cyclin D1-stimulated ER activity by wild-type P/CAF, but not by a mutant derivative that lacks HAT activity. (*A*) SAOS-2 cells were transfected with the reporter plasmids p(ERE)$_2$-TATA-luc (0.25 μg), pCMV-β-galactosidase (0.5 μg), and expression plasmids encoding the ER (50 ng), cyclin D1 (0 or 1.0 μg), plus FLAG-P/CAF or HAT(−) (0, 0.5, 1.0, 1.5, or 2.0 μg). To assess the effects of cyclin D1, P/CAF, and HAT(−) on the reporter in the absence of the ER, 2 μg of each coactivator was used. The respective empty vectors were used to make up a total of 3 μg DNA in each transfection. Where indicated, the cells were treated with estradiol (10 nM) for 24 hr. The fold activation is calculated relative to that of the ER alone, which is set to 1. The graph shows the averages + SEs from three independent experiments, each of which was done in duplicate. (*B*) Acetyl-transferase activity of P/CAF and the HAT(−) mutant. Immunoprecipitations using an anti-FLAG antibody were performed on lysates from COS cells transfected with plasmids encoding FLAG-tagged P/CAF or the HAT(−) mutant derivative. The precipitated proteins then were incubated with histones in the presence of ^{14}C-acetyl-CoA. Proteins were resolved by SDS/PAGE in a composite gel cast, with 15% acrylamide in the lower half and 10% acrylamide in the upper half, and subsequently transferred to a polyvinylidene difluoride membrane and exposed to film for 48 hr. (*C*) The association between cyclin D1 and P/CAF or the HAT(−) derivative. Immunoprecipitations were carried out with anti-FLAG antibodies on lysates from COS cells transfected with the indicated plasmids. Precipitates were analyzed by Western blotting for their cyclin D1 and FLAG-P/CAF content.

Because P/CAF also can bind to P300/CBP, SRC-1, and AIB1 (30), each of which can be recruited to the liganded ER and enhance estrogen-induced ER activity when overexpressed, it is conceivable that one or more of these may also be recruited to the P/CAF-cyclin D1-ER complex. Among the coactivators, P/CAF, P300/CBP, SRC-1, AIB1, and estrogen-bound ER, individual *in vitro* interactions have been demonstrated for every possible pair except SRC-1 and AIB1 (21, 25, 27, 30, 37, 49, 50). These results, taken together with the fact that neutralizing antibodies against P/CAF, SRC-1, and AIB1 have been shown to inhibit nuclear receptor activity, have led to the proposal of a model in which all the proteins are joined together in one complex (30). During the preparation of this manuscript, Zwijsen *et al.* (51) reported an interaction between cyclin D1 and the coactivators SRC-1 and AIB1. They also found that cyclin D1 could bring about an estrogen-independent recruitment of SRC-1 to the ER when the proteins were mixed together *in vitro*. Thus, cyclin D1 may be able to interact with multiple coactivators. The actual composition and geometry of the active estrogen- or cyclin D1-bound ER complexes cannot be determined from the available data at present. Such complexes may be subject to cell-lineage or signal-dependent variability. For example, it has been shown recently that in the epithelium of the rat mammary gland, SRC-1 is expressed only in the subset of cells that do not express the ER. Upon estrogen treatment, the expression of an estrogen-responsive target gene could be induced in the ER-containing cells, indicating that the ER can function in the absence of SRC-1 (52). It is also unclear whether or not SRC-1-, AIB1-, and p300/CBP-containing P/CAF complexes overlap with, or are mutually exclusive of, the SAGA-like P/CAF complexes. Each type of complex contains components that have been implicated in ER activation. Further studies will be required to understand which complexes are formed under which circumstances and with what types of biological effects.

Transcription appears to involve a multiplicity of contacts among the various components of an activation complex, perhaps to stabilize critical interactions or to trigger conformation-dependent activities (53). We suggest that cyclin D1 may stimulate the transcriptional activity of the ER by promoting or stabilizing an association between the ER and

P/CAF, thereby providing HAT activity and perhaps also forging or enhancing links to additional components of the transcriptional apparatus.

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