A Progesterone Receptor Co-activator (JDP2) Mediates Activity through Interaction with Residues in the Carboxyl-terminal Extension of the DNA Binding Domain*

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Progesterone receptor (PR) belongs to the nuclear receptor family of ligand-dependent transcription factors and mediates the major biological effects of progesterone. Transcriptional co-activators that are recruited by PR through the carboxyl-terminal ligand binding domain have been studied extensively. Much less is known about co-activators that interact with other regions of receptors. Jun dimerization protein 2 (JDP2) is a PR co-activator that enhances the transcriptional activity of the amino-terminal domain by increasing the α-helical content and stability of the intrinsically disordered amino-terminal domain. To gain insights into the mechanism of JDP2 co-activation of PR, the structural basis of JDP2-PR interaction was analyzed using NMR. The smallest regions of each protein needed for efficient protein interaction were used for NMR and included the basic region plus leucine zipper (bZIP) domain of JDP2 and the core zinc modules of the PR DNA binding domain plus the intrinsically disordered carboxyl-terminal extension (CTE) of the DNA binding domain. Chemical shift changes in PR upon titration with JDP2 revealed that most of the residues involved in binding of JDP2 reside within the CTE. The importance of the CTE for binding JDP2 was confirmed by peptide competition and mutational analyses. Point mutations within CTE sites identified by NMR and a CTE domain swapping experiment also confirmed the functional importance of JDP2 interaction with the CTE for enhancement of PR transcriptional activity. These studies provide insights into the role and functional importance of the CTE for co-activator interactions.
transient protein–protein interaction that relieves a repressive effect of the CTE on DNA binding (27–35).

The AF2 region of nuclear receptors interacts with the p160 family of steroid receptor co-activators through an LXLL motif that recognizes a specific complementary hydrophobic cleft in the well structured LBD. The structural basis for ligand-dependent interactions of p160 co-activators with AF2 has been well characterized by high resolution co-crystal structures of various LBD-LXLL peptide complexes (5, 36–38). Much less is known about proteins that interact with and mediate transcriptional activity of the NTD. Consistent with an intrinsically disordered region, a wide range of co-regulatory proteins has been reported to interact with the NTD (9, 10, 39). Studies have also demonstrated that the NTD of various steroid receptors interacts with components of the general transcription machinery (TATA binding protein, transcription factor IIH) or with the co-activator CREB-binding protein and that these interactions induce a more ordered folded state of the NTD (40–45). These results have led to the idea that protein-induced folding of the NTD mediates AF1 activity.

We previously identified Jun dimerization protein 2 (JDP2) as a PR-interacting protein that enhances the transcriptional activity of the NTD and can act in a manner independent of the LBD AF2 (46–48). JDP2 is a basic DNA binding domain plus leucine zipper (bZIP) protein that lacks an amino-terminal activation domain (49). Interaction of endogenous JDP2 and PR in T47D breast cancer cells was detected by co-immunoprecipitation assay, and the chromatin immunoprecipitation assay demonstrated a hormone-dependent co-recruitment of JDP2 and PR to the proximal PRE-containing promoter of an MMTV reporter gene stably integrated in T47D cells (47). Mapping studies identified the DBD plus CTE as the minimal JDP2 binding region within PR (46, 47). Results from circular dichroism, partial proteolysis, and functional mutagenesis experiments demonstrated that JDP2 interaction promotes a more ordered structure of the NTD in a manner that correlates with enhanced transcriptional activity of the NTD (48). Because JDP2 interaction occurs with the DBD and not directly with the NTD, this suggests that its effect on PR transcriptional activity is propagated through an interdomain communication between the DBD and the NTD.

The goal of this work was to further define the mechanism by which JDP2 interacts with PR to modulate transcriptional activity of the NTD. Several approaches were used to map functionally important JDP2 interaction sites in PR, including NMR chemical shift perturbations, and the effects of mutations on JDP2-PR protein interactions and PR transcriptional activity. Specific residues in a subregion of the CTE were defined as most critical for JDP2 binding and for co-activation of PR, whereas other residues in the CTE and core DBD appeared to undergo a conformational change or a change in their chemical environment. These data implicate a role for the CTE as a binding site for co-activator proteins and provide insights into the mechanism of interaction of co-activators that bind to regions of steroid receptors other than AF2 in the LBD.

**EXPERIMENTAL PROCEDURES**

**Plasmids**—PR expression vectors and reporter plasmids have been described previously including pHPR-B expressing full-length human PR-B under the control of the SV40 promoter. pRL-SV40 (Promega, Madison, WI) containing a cDNA (Rluc) encoding Renilla luciferase was used as an internal control reporter. A reporter gene construct PRETTATA-LUC was described previously (48). Amino-terminal glutathione S-transferase (GST)-tagged JDP2 constructs were constructed by PCR amplification of full-length cDNAs with primers containing unique 5′ and 3′ restriction sites for insertion into BamHI and EcoRI sites of pGEX-2T. JDP2 constructs included: full-length rat JDP2 (aa 1–163), JDP2 amino-terminal plus basic region (aa 1–96), JDP2 bZIP region (aa 69–137), JDP2 basic region (aa 74–96), and JDP2 leucine zipper region (ZIP) (aa 105–137). GST-tagged full-length rat JDP2 (aa 1–163; 18.8 kDa) was created from a pCDNA his-rJDP2 vector from A. Aronheim (Technion-Israel Institute of Technology). pCR3.1 rat JDP2, under the control of the cytomegalovirus promoter, was constructed by insertion of JDP2 cDNA into BamHI and EcoRI sites of the pCR3.1 vector (Invitrogen).

**Hormones, Antibodies, and Western Blots**—Progesterone was obtained from Sigma. Antibodies included a rabbit polyclonal prepared against the full-length rat JDP2 protein and a PR DBD polyclonal antibody as described previously (31). Proteins were separated on 12 or 15% SDS-polyacrylamide gel electrophoresis and analyzed by Western blotting as described previously (29, 31, 48). Detection was enhanced by chemiluminescence (Millipore).

**Bacterial Expression of Recombinant JDP2 and PR DBD**—JDP2 was overexpressed in *Escherichia coli* strain BL21 and cells were grown at 37°C to an A600 of 0.8 after protein expression was induced by 1 mM isopropyl β-D-thiogalactopyranoside for 4 h. Cells were harvested, and the pellet was resuspended in a detergent buffer (4 mM EDTA, 0.2 M NaCl, 1% deoxycholic acid, 1% Nonident P-40, 20 mM Tris-HCl, pH 7.5) containing lysozyme and DNase I. The clarified lysate was incubated with glutathione-Sepharose 4B (Amersham Biosciences) for 3 h at 4°C. After extensive washing of the resin with a high salt wash (3 M NaCl) and equilibration in thrombin cleavage buffer (20 mM Tris, pH 8.0, 250 mM NaCl, 10% glycerol, 1 mM DTT), JDP2 was cleaved from the resin with thrombin at 4°C for 12–16 h, concentrated, and further purified using a cation exchange Source 15S column (GE Healthcare). GST-tagged PR DBD was overproduced in *E. coli* strain BL21 (DE3) using the pGEX-2T overexpression system. Cells were grown at 37°C in Luria broth. When the A600 reached 0.8, protein expression was induced by the addition of 1 mM isopropyl β-D-thiogalactopyranoside, and cells were harvested after 4 h. The cells were lysed in a buffer containing 1 × BugBuster (Novagen), 5 mM DTT, and Benzonase and protease inhibitors, and the cell extract was cleared by centrifugation. The cell lysate was then incubated with glutathione-Sepharose beads for 3 h at 4°C, and the beads were washed repeatedly in a buffer containing 20 mM Tris, pH 7.5, 1 M NaCl, 100 mM ZnCl, 1 mM EDTA, 10% glycerol, and 1 mM DTT and then cleaved overnight in a buffer containing 20
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mm Tris, pH 7.5, 100 mm NaCl, 2.5 mm CaCl₂, 10% glycerol, 1 mm DTT, and 400 units of thrombin. Cleaved protein was loaded onto a Resource S cation exchange column (GE Healthcare, Upchurch) in a buffer containing 20 mm Tris, pH 7.5, 100 mm NaCl, 1 mm ZnCl₂, 10% glycerol, 1 mm EDTA, and 1 mm DTT, eluted with a linear salt gradient to 750 mm NaCl, and then dialyzed into the same buffer with 100 mm NaCl.

Cell Culture and Transfection—Cos-1 cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 5% fetal bovine serum (HyClone-Millipore, Billerica, MA). Cells were plated in 6-well dishes at a density of 1.6 × 10⁵ cells/well. At 24 h after plating, Cos-1 cells were transfected using Lipofectamine Plus reagents (Invitrogen) according to the manufacturer’s instructions. Following transfection, cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 5% charcoal-stripped fetal bovine serum for 24 h and then treated in the same medium with 10 nm progesterone.

In Vitro GST Pulldown Protein Interaction Assays—Bacterial cell lysates containing free GST protein or GST-JDP2 fusion proteins, as described previously (47), were incubated with 50 μl of a 50% slurry of glutathione-Sepharose resins (GE Healthcare) in phosphate-buffered saline for 1 h at 4°C. Resins were then washed three times (10 mm Tris-HCl, pH 7.8, 100 mm NaCl, 10% glycerol, 2 mm MgCl₂, 1 mm EDTA, 1 mm DTT, and 100 μg/ml bovine serum albumin), brought to a total volume of 250 μl in wash buffer, and incubated with PR DBD protein for 1 h at 4°C. Resins were then washed extensively, and the bound proteins were eluted with 2% SDS-β-mercaptoethanol sample buffer and detected by Western immunoblotting. In peptide competition assays, 1 μg of purified PR DBD-CTE was incubated with CTE peptide or scrambled CTE (sCTE).

Cell-based Receptor Transactivation Assays—Cos-1 cells were plated at 1.6 × 10⁵ cells/well in six-well culture dishes. At 24 h, cells were transfected with constitutively active pRL-SV40 (Promega) Renilla luciferase expression vector as an internal control for transfection efficiency, PRE-TATA-Luc (Promega) and varied amounts of pCR3.1-JDP2 expression vectors, and varying amounts of pCR3.1-JDP2 constructs or free GST were immobilized to glutathione-Sepharose resins and incubated with a PR DBD construct that also contains amino acids 362–641 of the CTE (PR DBD-CTE641). PR DBD-CTE641 bound to all of the GST-JDP2 constructs with the exception of the leucine zipper (Fig. 1C). Thus, the smallest region of JDP2 that interacts with PR, various GST-JDP2 constructs (Fig. 1B) were used in pulldown assays including full-length JDP2 (aa 1–163), the amino terminus plus basic region (aa 1–96), basic leucine zipper region (aa 69–137), basic region alone (aa 74–96), and leucine zipper region alone (aa 105–137). GST-JDP2 constructs or free GST were immobilized to glutathione-Sepharose resins and incubated with a PR DBD construct that also contains amino acids 632–641 of the CTE (PR DBD-CTE641). PR DBD-CTE641 bound to all of the GST-JDP2 constructs with the leucine zipper (Fig. 1C).

RESULTS

JDP2 Interaction Perturbs Residues in the Core DBD and CTE as Detected by NMR—Our previous studies mapped the region of PR that binds JDP2 to the PR DBD plus CTE and the bZIP as the minimal portion of JDP2 (47). To further narrow the region of JDP2 that interacts with PR, various GST-JDP2 constructs (Fig. 1B) were used in pulldown assays including full-length JDP2 (aa 1–163), the amino terminus plus basic region (aa 1–96), basic leucine zipper region (aa 69–137), basic region alone (aa 74–96), and leucine zipper region alone (aa 105–137). GST-JDP2 constructs or free GST were immobilized to glutathione-Sepharose resins and incubated with a PR DBD construct that also contains amino acids 632–641 of the CTE (PR DBD-CTE641). PR DBD-CTE641 bound to all of the GST-JDP2 constructs with the leucine zipper (Fig. 1C). Thus, the smallest region of JDP2 that interacted with the PR DBD-CTE641 was the basic domain. Although smaller domains or fragments are theoretically more amenable for NMR studies, the basic region alone was less stable during purification than other fragments. Therefore, the bZIP was expressed and purified for NMR studies.

Because JDP2 bZIP and PR DBD-CTE641 are small and can be expressed in bacteria and purified as soluble proteins at high concentrations (5 mg/ml), the PR-JDP2 complex is amenable to NMR analysis. ¹³C ¹⁵N-labeled PR DBD-CTE641 was produced for NMR experiments as described under “Experimental Procedures.” This construct is similar to that used previously for crystallography, which was sufficient for visualization of the CTE interaction with the minor groove of DNA (21). A series of NMR spectra were recorded including HSQC, HNCA, TOCSY-CONH, TOCSY-HSQC, CACBCONH, and HAHCBCONH. Backbone chemical shift assignments were made, and the labeled resonance assignments of the amide nitrogen and hydrogen atoms for each amino acid are shown in the ¹⁵N-HSQC spectrum (Fig. 2A and supplemental Table 1).

Comparison of the chemical shift values with the predicted chemical shift values—

NMR Studies—GST-tagged ¹³C ¹⁵N-labeled PR DBD-CTE (aa 562–641) was expressed in E. coli strain BL21 (DE3) using the pGEX-2T expression system (Novagen). Cells were grown at 37°C in a minimal medium containing 1g of ¹⁵NH₄Cl and 2g of ¹³C-glucose in 1× M9 minimal medium supplemented with 2 mm MgSO₄ and 0.1 mm CaCl₂/liter medium. When the A₆₀₀ reached 0.5, protein expression was induced by the addition of 0.5 mm isopropyl-β-D-thiogalactopyranoside, and cells were harvested after 4 h. The protein was purified as described above. The collected fractions were pooled and dialyzed into a buffer containing 20 mm Tris, pH 7.5, 100 mm NaCl, 1 mm ZnCl₂, 10% glycerol, 1 mm EDTA, and 1 mm DTT and concentrated to 400 μM. The NMR sample was combined with 10% D₂O and two granules of 2,2-dimethyl-2-silapentane-5-sulfonic acid as a reference. NMR spectra were recorded at 15°C on a Varian 600-MHz spectrometer using a (5-mm) triple-resonance (¹H/¹³C/¹⁵N) pulsed field gradient cold probe. The following spectra were recorded: HSQC, HNCA, TOCSY-CONH, TOCSY-HSQC, CACBCONH, and HAHCBCONH. NMR data were processed using NMRPipe (50) and analyzed using SPARKY (51). ¹H chemical shifts were referenced to 2,2-dimethyl-2-silapentane-5-sulfonic acid, and ¹⁵N and ¹³C chemical shifts were referenced indirectly from the gyromagnetic ratios.
shifts from the crystal structure of the PR DBD-DNA complex (21) demonstrates that there are no major conformational differences between the free and DNA-bound protein with the exception of a few residues in the β-hairpin, the end of helix 1, and the CTE (52–56) (supplemental Fig. 1). This implies that the DBD-CTE in solution takes on a slightly different conformation than the DBD-CTE bound to DNA. To investigate this further, the chemical shift assignments for PR DBD-CTE641 were submitted to an online structure prediction server, THRIFTY (56, 57). The predicted three-dimensional structure of DBD-CTE641 in solution is similar to the crystal structure of PR DBD-CTE641 bound to DNA (21), except for an altered position of the CTE. These data implicate the CTE as a flexible region that is capable of adopting a different conformation when bound to a partner macromolecule (supplemental Fig. 2).

Chemical shift perturbations were used to identify potential sites of PR that interact with JDP2. As increasing amounts of unlabeled JDP2 bZIP were titrated into 15N-labeled PR DBD-CTE641 and successive HSQC spectra were recorded, chemical shift changes in several resonances were noted. The residues that shift or disappear with the addition of JDP2 bZIP are shown in the overlay and enlargement of the HSQC spectra in Fig. 2B. For example, resonances corresponding to Gly635 and Gly636 in the CTE disappeared upon the addition of JDP2, whereas residues Arg637 and Lys638 underwent a chemical shift change (Fig. 2B). Chemical shift changes were also observed in the core DBD second zinc finger, most notably Gly585 at the beginning of the DNA recognition helix and Arg593 at the end of the helix, a hydrophobic patch around Met595 and Gly597, and Asp612 and Lys613 in the dimerization box (Fig. 2B).

Using the chemical shift assignments made with the double-la-
beled PR DBD-CTE641, potential JDP2 binding sites were iden-
tified as chemical shift differences between the free and bound
forms of the protein. The relative chemical shift differences per
residue superimposed on a schematic of the PR DBD-CTE641
(Fig. 3A) and a color map of the three-dimensional crystal
structure (21) (Fig. 3B) illustrate the regions of PR influenced by

FIGURE 3. Overlay of NMR chemical shift changes in the presence of JDP2 on the structure of the PR DBD-CTE. A, chemical shift changes between PR
DBD-CTE641 alone and PR DBD-CTE641 with JDP2 bZIP were quantitated by \(0.5(\Delta NH^2 + \Delta N^2)/25\) \(^{1/2}\) (74). Larger differences indicate more of a shift change upon
the addition of JDP2 bZIP. Resonances with the largest perturbations by JDP2 are colored in red. D box, dimerization box. B, color-coded map of PR DBD-CTE
crystal structure (21) showing areas of chemical shift changes in the presence of JDP2 bZIP; red represents the greatest change, and gray represents the least
change.
JDP2 bZIP binding. The greatest changes in chemical shifts occurred in helix 1 of the core DBD, the dimerization box, and the CTE. In contrast, helices 2 and 2’ of the core DBD showed little change in backbone chemical shift, indicating that these regions do not interact with JDP2 bZIP or undergo a change in chemical environment as a result of JDP2 bZIP binding.

NMR-guided Mutagenesis Shows That Specific Residues in the CTE Are Required for PR Interaction with JDP2—To examine the involvement of the PR DBD-CTE residues perturbed in the NMR spectra for JDP2 binding, we analyzed the effects of specific amino acid substitutions in the PR DBD-CTE_{641} construct by GST pulldown assays. Amino acids in the PR DBD-CTE_{641} that exhibited chemical shift changes in the presence of JDP2 were substituted only if the change was predicted to not disrupt global protein folding based on Ramachandran angles. Substitutions of core DBD residues had little (G585A and R593Q) or minimal (K613Q) effect on JDP2 binding, as did substitutions in two CTE residues (G635A and G636A) that disappeared in the NMR spectrum upon the addition of JDP2 (Fig. 4A). These data suggest that these amino acids undergo a conformational change upon JDP2 binding as opposed to representing residues within direct contact sites. However, substitution of two other residues in the CTE (R637A/K638A), which undergo chemical shift changes, abolished JDP2 binding (Fig. 4B). Truncation mutations of the CTE constructs were also examined for their effects on binding to JDP2 (Fig. 5A). JDP2 bound with equal efficiency to CTE constructs deleted from aa 650 to 648 and to 641, whereas no binding was detected with further deletion of the CTE to aa 632 (Fig. 5B). These data collectively indicate that residues in the CTE closest to the core DBD (aa 632–641) are most important for binding JDP2 and that residues in the core are dispensable.

To further explore the role of the CTE in binding JDP2, a synthetic peptide corresponding to CTE sequence aa 636–654 (Fig. 6A) was tested for its ability to compete for JDP2 binding to PR. The CTE peptide inhibited the interaction between JDP2 bZIP and DBD-CTE_{650} in a GST pulldown assay, whereas a scrambled control peptide with the same amino acid composition had little effect (Fig. 6B). This result indicates that the CTE alone is sufficient for binding JDP2 and that a specific sequence is required. Taken together, these results indicate that the CTE plays a critical role in protein interaction with the
JDP2. Specifically, Arg<sup>637</sup> and Lys<sup>638</sup> are more crucial for JDP2 binding, whereas Gly<sup>635</sup> and Gly<sup>636</sup>, which had large changes in chemical shift, are predicted to be involved in the effects of JDP2 on dynamics or conformation of the CTE.

**FIGURE 6.** The PR CTE alone is sufficient for binding JDP2-PR binding. A, sequences of synthetic peptides used to compete for JDP2-PR binding. The CTE peptide is a 19-mer corresponding to aa 632–656 of the PR CTE. sCTE (scrambled) is a 19-mer containing the same amino acid composition as the CTE except that the sequence was randomly scrambled. B, PR DBD-CTE<sub>650</sub> was incubated with either free GST or GST-JDP2-bZIP immobilized to glutathione-Sepharose resins in the presence of varying amounts of either CTE or sCTE peptides. Bound protein along with input PR DBD-CTE<sub>650</sub> were eluted after washing resins and detected by immunoblotting with a PR DBD-specific antibody.

**FIGURE 7.** Functional requirement of CTE for JDP2 enhancement of PR-mediated gene transcription in cells. A, mutations in CTE enhance progesterone-dependent transcription activity of PR in the absence of ectopically expressed JDP2. Cos-1 cells were transiently co-transfected with a PRE<sub>2</sub>-TATA-luciferase reporter gene and an expression plasmid for wild type PR-B or PR-B containing substitution mutations in the CTE (R637A/K638A). Cells were treated without (vehicle) or with progesterone (Prog) (10 nM) for 48 h and assayed for luciferase activity as described under "Experimental Procedures." Results were calculated as relative luciferase activity in vehicle versus progesterone treated cells and are average values ± S.E. (error bars) from three independent experiments. Paired type 1 Student’s t test analysis was used to determine statistical significance for the difference of progesterone induction between PR-B and PR-B R637A/K638A; p < 0.05. The inset is an immunoblot of cell extracts used in luciferase assays with a PR-specific antibody (1294) to detect relative levels of WT PR-B and PR-B R637A/K638A mutant protein expression (PR-B is 118 kDa). B, mutations in CTE attenuated JDP2 enhancement of PR transcriptional activity. Cos-1 cells were co-transfected as in A above with PR-B or PR-B CTE (R637A/K638A) and PRE-TATA-luc except in the absence (empty vector) or presence of varying amounts (25, 50, 75 ng) of JDP2 expression plasmids. Results were calculated as fold JDP2 stimulation of PR-mediated transactivation of luciferase reporter gene activity in the presence of progesterone by setting relative luciferase activity of each receptor in the absence of transfected JDP2 to 1.0. Data represent average values ± S.E. from three independent experiments. Paired type 1 Student’s t test analysis was used to determine statistical significance between the effect of JDP2 on PR-B and PR-B R637A/K638A mutant protein expression (PR-B is 118 kDa). C, swapping of TR for PR CTE eliminated JDP2 stimulation of luciferase activity. Cos-1 cells were co-transfected as in B except with PR-B or a PR-TR chimera consisting of a swap within full-length PR-B of the CTE from TR for PR CTE. Results were calculated as fold increase of JDP2 stimulation in the presence by setting relative luciferase activity of each receptor in the absence of transfected JDP2 to 1.0. Data represent average values ± S.E. from three independent experiments. Paired type 1 Student’s t test analysis was used to determine statistical significance between effects of JDP2 on PR-B and PR-TR chimera; p < 0.05.
the PR-B mutant (R637A/K638A) as compared with that of the wild-type PR (Fig. 7B).

To further analyze the functional role of the CTE in cells, a receptor chimera consisting of the CTE of PR-B, replaced with the CTE of the thyroid receptor (TR), was transfected into Cos-1 cells in the absence and presence of increasing amounts of JDP2. Ectopic expression of JDP2 had no effect on progestrone-induced transactivation mediated by the PR/HR8528 TR chimera as compared with wild type PR (Fig. 7C). Thus, the CTE of PR specifically is required for functional response to JDP2 in cells.

**DISCUSSION**

The results presented here provide insights into the mechanism by which JDP2 interacts with PR to stimulate its transcriptional activity. The basic DNA binding domain is the minimal region of JDP2 required for protein interaction with PR. Interaction is not mediated directly through the leucine zipper. NMR spectroscopy coupled with the effects of specific amino acid substitutions on PR activity have defined residues in a specific region of the PR CTE as most critical for JDP2 binding and for functional response to JDP2 as a co-activator. Other residues in the CTE and core DBD of PR that were perturbed in the NMR spectra by JDP2, but were not required for JDP2 binding, undergo either conformational changes or alteration in their chemical environment due to other residues that are affected structurally by binding to JDP2. JDP2-PR CTE interaction exhibits sequence specificity, as shown by the peptide competition assay, and was necessary for JDP2 co-activation of PR-mediated transcription in cells. These data, along with previous reports that the CTE is important for binding DNA and for interaction with HMGB proteins (28–31, 34), implicate the CTE as an important modulatory region of steroid receptors (Fig. 8).

Of the residues in the CTE that exhibited chemical shift changes in the NMR spectra in the presence of JDP2 (Fig. 2), only mutations in Arg637 and Lys638 abolished JDP2 binding; mutations in Gly635 or Gly636 had no effect (Fig. 4). Based on truncation mutations, sequences of the CTE between aa 632 and 641 that encompasses the residues were required for JDP2 binding, suggesting that this subregion constitutes a JDP2 binding site (Fig. 5) and that more than one site is required. The fact that mutations of Gly635 and Gly636 did not affect JDP2 binding suggests that the chemical environment of these residues was perturbed by JDP2 binding to residues elsewhere (possibly Arg637 and Lys638). Interestingly, the CTE sites implicated in direct JDP2 binding are also involved in DNA binding (21), and another subregion of the CTE (aa 641–651) was demonstrated previously to be required for binding the co-regulator protein, HMGB (31). These data suggest that the CTE is dynamic and capable of binding protein or DNA within distinct and overlapping subregions (Fig. 8).

Most steroid receptors have at least one glycine residue between the Gly-Met (GM) boundary of the core DBD and the CTE that could provide the flexibility for the CTE to adopt different conformations. In the recent crystal structure of a full-length PPAR-γ-RXR-α heterodimer bound to DNA, the CTE of PPAR-γ was observed to make contact with DNA, whereas the CTE of RXR-α makes dimer contacts with PPAR-γ. The RXR-α CTE in particular lacks secondary structure, giving it flexibility that may be an adaptive feature to allow for more promiscuous protein interactions and DNA binding (58). Flexibility of the CTE has also been demonstrated in high resolution crystal structures of the orphan nuclear receptor ERR-2. The CTE of ERR-2 was unstructured in the absence of DNA and adopted secondary structure when bound to DNA (16, 22). The recently
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reported computational methods of Wishart et al. (56) that generate protein three-dimensional structure from NMR chemical shift assignments and protein sequence were used to analyze the PR DBD-CTE<sub>641</sub>. This analysis indicated a change in conformation of the CTE in solution versus the crystal structure of PR DBD-CTE<sub>641</sub> bound to DNA. (supplemental Fig. 2).

Previous work from our laboratory demonstrated that JDP2 functions as an NTD co-activator independent of AF2 in the LBD (47, 48). The current work suggests that the CTE plays a critical role for both JDP2 binding and co-activator function.

Cell-based assays using CTE point mutations (R637A/K638A) showed a significant decrease in JDP2 enhancement of PR activity. However, some effect of JDP2 still occurred, suggesting that other residues in the CTE are also involved. The functional requirement of the CTE for JDP2 co-activation was also shown by analysis of a PR-TR chimera that contains the CTE of TR in place of the PR CTE. In contrast to steroid receptor CTEs, which are flexible with little secondary structure, the CTEs of TR and other non-steroid nuclear receptors are reported to exist as a stable α-helix (7). We previously showed that the CTE is a binding site for HMGB-1 and that it mediates the effects of HMGB-1 to increase DNA binding of PR. The TR CTE does not bind HMGB, and a PR-TR CTE chimera also failed to respond to HMGB, whereas the reverse TR-PR CTE chimera, containing the CTE of PR in place of TR, did respond to HMGB-1 (29, 30).

These data suggest fundamental differences in the properties of TR and PR CTEs. Consistent with this conclusion, JDP2 had no detectable effect on the transcriptional activity of a PR-TR CTE chimera, indicating that both the sequence and the structure of the PR CTE are critical for PR co-activation by JDP2.

How the short CTE can be a binding site for DNA and protein is an interesting and unanswered question. The CTE appears to be flexible and capable of interacting with multiple partners in a dynamic and transient fashion. Based on the results of the present work and earlier studies of the CTE interaction with the minor groove of DNA and HMGB, we propose that the CTE is a short IDP region capable of interacting with DNA and other proteins (Fig. 8). Many transcription factors have IDP regions, indicating an important regulatory role for these disordered regions (11–15). A recent model proposed that intrinsic disorder in multidomain proteins can maximize the ability to allosterically couple two domains and that the ability to propagate the effects of binding partners is determined by the energetic balance within the protein due to degenerate requirements for coupling, rather than by a mechanical pathway linking two protein domains (59). We have previously shown that the primary effect of JDP2 on the activity of PR is associated with increased folding of the NTD (48). We propose that the CTE is involved in coupling between the DBD and NTD domains through conformational changes in the CTE induced by JDP2 binding.

Structural and biochemical studies of bZIP proteins in complexes with other proteins and DNA provide a working model for the structural basis for JDP2-PR interactions. Fos/Jun heterodimer interaction with the transcription factor nuclear factor of activated T-cells (NFAT) is mediated by contacts in the fork between the basic region and the coiled-coil region of the Jun subunit, as well as the coiled-coil region of both Fos and Jun (60, 61). However, in the crystal structure of ATF-2/Jun and IRE-3 bound to DNA, ATF-2 interacts with two loops, L1 and L3, of IRE-3 through only its basic region (62). The basic region of bZIP proteins contains a distinct surface that directly contacts DNA and another involved in mediating interaction with co-activators. For example, the selectivity of Chameau and MFB1 histone acetylases for bZIP proteins is mediated by residues in the basic region that lie on the opposite surface from the residues that contact DNA (63).

In addition to JDP2, other co-activators that bind to the DBDs of steroid receptors have been described. The small nuclear RING finger protein (SNURF) binds to the DBD of hormone-activated androgen receptor (AR), ER, and PR to enhance steroid receptor-dependent transcription and has been suggested to act as a bridging factor between steroid receptors and other transcription factors (64–66). GT198 interacts with many NRs through their DBDs to enhance transcription, including ERα and -β, TRβ1, androgen receptor (AR), glucocorticoid receptor (GR), and PR (67). The co-activator and histone acetylase PCAF (p300/CREB-binding protein-associated factor) interacts, independently of p300/CREB-binding protein, with an RXR-RAR (retinoic acid receptor) heterodimer through the DBD of either receptor (68). The ERα-associated protein pp32 has been shown to interact with the ER DBD in a hormone-independent manner and to increase ERα-ERE complex formation but acts to repress ERα-mediated transcription (69). pp32 was also shown to decrease the acetylation of ERα both in vitro and in cells, which may play a role in its co-repressor activity. More recently, proliferating cell nuclear antigen (PCNA) was shown to interact with the DBD of ERα and to enhance receptor-DNA interaction in vitro, leading to increased basal transcription of endogenous estrogen-responsive genes. Interestingly, it was found that proliferating cell nuclear antigen interacts with the CTE region of the ERα DBD (70). Additionally, DNA has been reported to act as ligand and to induce conformational changes in the DBD and/or transcription activation surfaces of NRs, thereby influencing co-regulatory protein interactions and NR function (71–73).

Despite the number of DBD-interacting co-factors reported, there has been little work to define interaction sites within the DBD or mechanisms of co-activation. Because most of the surfaces of the core DBD are involved in interactions with DNA or dimerization, it is most likely that the unstructured CTE is a protein interaction site for many other co-regulators.

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