Organochlorine-mediated potentiation of the general coactivator p300 through p38 mitogen-activated protein kinase

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**Abstract**

Potentiation of transcription coactivators by specific signaling pathways is critical for cell fate decisions in response to extracellular environmental cues. Here, we examine the transcriptional coactivator p300, whose acetyltransferase activity is regulated by extracellular signaling events. We show that organochlorine pesticides stimulate p300 activity through the p38 mitogen-activated protein kinase (MAPK). In the presence of the p38 inhibitor SB203580, p300 and the general coactivator p300 acetylate the transcription factor Sp1, leading to its potentiation as a general transcription factor. These results suggest that p38 signaling plays a large role in the regulation and activity of p300.

**Introduction**

Nuclear transcription factors such as activator protein-1 (AP-1) and the estrogen receptor (ER) bind specific DNA response elements located in the promoter regions of target genes, driving transcription. Initiation of transcription requires the recruitment and binding of coactivators to specific regions located within the activation domains of the nuclear factors. For example, when stimulated, the AP-1 components c-Jun (Jun oncogene) and c-Fos (FBJ osteosarcoma oncogene) recruit the p300/cyclic adenosine monophosphate response element binding protein and Elk1 and potentiate gene expression through cyclic adenosine monophosphate and hypoxia response elements. Because p300 is phosphorylated and potentiated by the p38 mitogen-activated protein kinase, we used p38 MAPK signaling cascade to potentiate p300. Our results obtained from molecular and pharmacological inhibitors in addition to in vitro kinase assays strongly suggest that p38 phosphatase and potentiates the p300 coactivator. To test this hypothesis, we used DDT as a pharmacological tool to test the ability of the p38 MAPK signaling cascade to potentiate p300.

**Materials and methods**

**Chemicals**

p-o-Dichloro-phenylacetonitrile, p-o'-Dichlorodiphenyldichloroethane and p-p'-dichlorodiphenyl acetic acid were purchased from AccuStandard (New Haven, CT); 3,4-Dichlorodiphenyl dichloroethane was purchased from Sigma and dissolved in Dulbecco’s modified Eagle’s medium (DMEM). U0126 [MAPK/ERK kinase 1/2 inhibitor] was purchased from Promega (Madison, WI). SB203580 (p38 inhibitor) was purchased from Calbiochem (San Diego, CA). All pharmacological inhibitors were dissolved in DMSO.

**Plasmids**

Cyclic adenosine monophosphate response element-luciferase (pCRE-luc) was purchased from Clontech (Palo Alto, CA). Hypoxia response element-luciferase (pHRE-luc) was generously donated by Barbara S. Beckman (Tulane University, New Orleans, LA 70112, USA). These authors contributed equally to this work and should both be considered first authors.

**Abbreviations:** AP-1, activator protein-1; CA-MKK, constitutively active dimethyl sulfoxide; DN, dominant negative; ER, estrogen receptor; ERK, extracellular-signal regulated kinase; ERK1/2 inhibitor was purchased from Promega (Madison, WI). Tetradecanoyl-13-phorbol acetate (PMA) was purchased from Sigma and dissolved in Dulbecco’s modified Eagle’s medium (DMEM). U0126 [MAPK/ERK kinase 1/2 inhibitor] was purchased from Promega (Madison, WI). SP600125 [c-Jun N-terminal kinase (JNK) inhibitor] was purchased from BIOMOL Research Laboratories (Plymouth Meeting, PA). SB203580 (p38 inhibitor) was purchased from Calbiochem (San Diego, CA). All pharmacological inhibitors were dissolved in DMSO.

**Acknowledgements:**

The authors are grateful to Drs. John A. C. Nusgens and Gregory J. Mesko for critical reading of the manuscript. This work was supported by grants from National Institutes of Health (NIH) 5R01CA120591, 5P50CA096551, and 5R01ES016301, and the Robert Wood Johnson Foundation Clinical Scholars Program (to M.B.).
University). pFR-Luc, pFA2-CREB (GAL4-CREB), pFA2-EIk1 (GAL4-EIk1) and pFC-MEK1 [constitutively active mitogen-activated protein kinase kinase (CA-MKK) 1] were purchased from Stratagene (La Jolla, CA). CMV-GAL4 (negative control), GAL4-p300 full length, GAL4-p300 (amino acid: 1–243) and GAL4-p300 (amino acid: 1–743) were generous gifts from Erik Flemington (Tulane University). GAL4-p300 (amino acid: 1–596), GAL4-p300 (amino acid: 744–1571), GAL4-p300 (amino acid: 1572–2414), pGEX-p300N (amino acid: 1–596), pGEX-p300M (amino acid: 744–1571) and pGEX-p300C (amino acid: 1572–2414) were kind gifts from Yang Shi (Harvard University). pcDNA3.1 expression vector was purchased from Invitrogen (Carlsbad, CA). pcDNA3-CA-MKK5 (constitutive active) and dominant-negative (DN)-ERK2 were gifts from Jin-Dwan Lee (Scripps Research Institute). pcDNA3-CA-M KK6 (constitutive active) and pcDNA3-CA-MKK7 (constitutive active) were kind gifts from Jiahui Han (Scripps Research Institute). JNK1 and p38 MAPK DN mutants (DN-JNK1 and DN-p38) were kindly provided by Roger Davis (University of Massachusetts Medical School). Empty glutathione S-transferase (GST) expression vector was purchased from Amersham Biosciences (Piscataway, NJ).

Cell culture and transient transfection
Human embryonic kidney (HEK) 293 cells were grown as described previously (18–20). These cells are ER negative and easily transfectable. Therefore, we can use them to study ER mutants without a wild-type background. Cultures of cells were transferred to phenol red-free DMEM supplemented with 5% dextran-coated charcoal-treated fetal bovine serum (FBS), essential and non-essential amino acids (Invitrogen, Carlsbad, CA), cat. # 11100-306 and 11140-035, respectfully), sodium pyruvate and penicillin–streptomycin for 24 h prior to plating. Cells were plated at a density of 5 × 10^5 cells per well in 24-well plates (~80% confluency) and maintained for an additional 24 h in DMEM with 5% dextran-coated charcoal–FBS.

For quantitative real-time polymerase chain reaction analysis, MCF-7 breast cancer cells were plated in 10 cm^2 plates for 24 h at ~80% confluency. Cells were grown for 48 h in phenol red-free media containing 5% charcoal-stripped FBS. Cells were then incubated for 18 h in the presence of DMSO (vehicle), 50 μM DDT, 10 μM DDT or DMSO and RWJ. Cells were harvested, and total RNA was extracted using the RNAeasy mini kit from Qiagen (Valencia, CA). Complementary DNA (cDNA) was prepared from 1 μg of total RNA using Bio-Rad’s (Hercules, CA) cDNA synthesis kit. Reverse transcription–polymerase chain reaction assays were assembled in 96-well plates using 5 μl of a 1:10 dilution of the synthesized cDNA, 0.1 μg of a 1:1 mixture of forward and reverse primers and 1× SYBR-Green solution (Bio-Rad).

For response element assays, HEK 293 cells were then transfected for 5 h with 100 ng of response element-luciferase using FuGENE 6™ lipofection reagent (Roche, Indianapolis, IN) according to the manufacturer’s protocol.

For GAL4 one-hybrid assays, 50 ng of pFR-Luc was transfected with FuGENE 6™ lipofection reagent according to the manufacturer’s protocol for 6 h in combination with 25 ng of GAL4-fusion protein construct, with or without 100 ng empty expression vector or CA-MKK construct. Cytomelagovirus promoters drove all expression vectors.

For DN experiments, HEK 293 cells were transfected with FuGENE 6™ lipofection reagent according to the manufacturer’s protocol for 24 h using 50 ng of pFR-Luc and 10 ng of GAL4-p300 full length in conjunction with 0, 30, 150 or 150 ng of DN mutant plasmid. Total DNA volume was brought up, if necessary, using empty pcDNA3.1 expression vector. Cytomelagovirus promoters drove all DN mutant expression vectors.

Reporter gene assay
For all luciferase assays, transfected HEK 293 cells were incubated for 18–24 h in DMEM with 5% dextran-coated charcoal–FBS in the presence of vehicle or various chemicals as described previously (18,20). Where indicated, kinase inhibitors were added 1 h prior to the addition of DMEM, PMA or DDT metabolites and maintained during the remainder of the incubation period. Kinase inhibitor concentrations were chosen based on non-toxic levels, published concentration of drug resulting in 50% inhibition values from the manufacturers, previous experiments demonstrating inhibition of known MAPK-signaling pathways (21). PMA was used as a positive control as our lab has shown previously that 20 ng/ml PMA gives significant activation of protein kinase, downstream MAPKs and AP-1 activation (22). In our results, we have shown the data from treatments using 10–50 μM DDT and its metabolites, which gave significant AP-1 activity as demonstrated previously (18,20). Finally, cells were harvested and luciferase activity was measured using 30 μl of cell extract and 100 μl of Luciferase Assay Substrate (Promega) in a Berthold AutoLumat Plus luminometer. The data shown are an average of at least three independent experiments with two replicates.

GST-fusion protein purification
The GST and GST-coactivator fusion proteins were generated using pGEX expression vectors transformed into BL21 star cells (Invitrogen). Bacteria were grown overnight in luria broth supplemented with 50 μg/ml ampicillin at 37°C with shaking. The following morning, bacteria were diluted 1:100 in fresh luria broth-supplemented media and grown at 37°C to an A600nm = 0.5–2. Protein expression was then induced for 3 h with 0.1 M isopropyl-β-D-thiogalactopyranoside. After induction, cells were collected by centrifugation at 7500 g for 10 min at 4°C. The supernatant was discarded; the pellet was resuspended in cold 1× phosphate-buffered saline supplemented with 1 mM phenylmethlysulfonyl fluoride and 10 μM protease inhibitor cocktail for use with bacterial cell lysates (Sigma). Resuspensions were frozen overnight at ~8°C. The following morning, suspensions were thawed and then sonicated mildly twice for 45 s. A total of 20% Triton X-100 was added to the suspensions to a final concentration of 1% and mixed gently for 30 min to help solubilize the fusion proteins. The suspensions were then centrifuged at 12 000g for 10 min at 4°C. The supernatants were transferred to fresh tubes and GST-fusion proteins were purified using the Bulk GST Purification Module (Amersham Biosciences) according to the manufacturer’s protocol.

In vitro kinase assay
Roughly, 3–5 μg of eluted purified GST-fusion protein or 200 ng of purified MAPK-activated protein kinase-2 (Upstate Biotechnology, Lake Placid, NY) was then incubated for 30 min at 30°C with shaking with 0.06 U activated p38 (Upstate Biotechnology) in the presence of magnesium/adenosine triphosphate cocktail containing [γ-32P] (Upstate Biotechnology) according to the manufacturer’s protocol. Reactions were stopped by the addition of 20 μl 2× sodium dodecyl sulfate sample buffer containing 1 M phenylmethylsulfonfyl fluoride, protease inhibitor cocktail, phosphatase inhibitor cocktail (Sigma) and β-mercaptoethanol and boiling samples for 5 min. Samples were analyzed by 4–12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (Invitrogen), stained with coomassie blue to monitor expression and subjected to autoradiography.

Western blot
Western blots were performed as described previously using ~50 μg of crude bacterial lysate or 10 μg of purified GST-fusion proteins analyzed by 4–12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Proteins were transferred to nitrocellulose membranes and probed using a 1:1000 dilution of goat anti-GST antibody (Amersham Biosciences) in blocking solution followed by rabbit anti-goat peroxidase-labeled antibody (1:2500 dilution in blocking solution) (Kirkegaard and Perry Laboratories, Gaithersburg, MD).

Fig. 1. DDT stimulates p38-mediated gene transcription. MCF-7 cells were incubated for 18 h with either vehicle, 50 μM DDT (solid bar) or DDT plus increasing amounts of RWJ67657 (0.1, 1.0 or 10 μM). Total RNA was collected from triplicate samples, followed by cDNA synthesis and quantitative real-time polymerase chain reaction analysis of the Fra-1 transcript. Value for vehicle set to 1.0. *Indicates statistical significance (P < 0.05).
Statistical analysis
Data were analyzed using one-way analysis of variance and post-hoc Tukey’s multiple comparisons with GraphPad Prism, Version 3.02 (GraphPad Software, La Jolla, CA). Statistically significant changes were determined at the \( P < 0.05 \), \( P < 0.01 \) or \( P < 0.001 \) level as indicated for each figure or table.

Results

**DDT stimulates p38-mediated gene transcription**

We have shown previously that DDT stimulates p38 phosphorylation and activation (18). To show that this activation culminates in specific p38-mediated gene transcription, we utilized quantitative real-time polymerase chain reaction analysis of the \( Fra-1 \) gene in MCF-7 cells after DDT incubation. \( Fra-1 \) is a heterodimeric partner of the AP-1 transcription complex, and its expression has been shown to be upregulated by p38 MAPK (23,24). DDT increased the transcription of \( Fra-1 \) by 5-fold over vehicle alone (Figure 1). The selective p38 inhibitor RWJ67657 greatly reduced DDT-stimulated \( Fra-1 \) expression (Figure 1). These data provide a direct link between DDT-stimulated p38 activation and p38-mediated gene transcription.

**DDT and its metabolites stimulate multiple promoter response elements and nuclear transcription factors**

Because DDT-induced p38 activity stimulates the AP-1 complex post-translationally (18), we hypothesized that DDT targets a more general transcriptional mechanism. To determine if DDT stimulation of AP-1 was a selective process, we tested whether DDT treatment could stimulate other promoter response elements and transcription factors. Using luciferase reporter gene assays, we demonstrate in HEK 293 cells that, similar to AP-1 sites, DDT and its metabolites also target cyclic adenosine monophosphate response element and hypoxia response element sites (Figure 2A and B). In addition, GAL4 one-hybrid analysis showed that DDT and its metabolites potentiate the nuclear transcription factors CREB and Elk1 (Figure 2C and D). In all cases, the active metabolites (\( o,p' \)-DDT, \( p,p' \)-DDT and \( o,p' \)-dichlorodiphenyldichloroethane) stimulated luciferase expression, whereas the inactive metabolites (\( p,p' \)-dichlorodiphenyl acetic acid and \( p,p' \)-dichlorodiphenyl ethanol) had no effect. These experiments indicate that DDT signaling targets a pathway shared by multiple transcription factors/response elements.

![DDT and its metabolites stimulate multiple response elements and nuclear transcription factors](image-url)
The transcriptional coactivator p300 is stimulated after DDT metabolite treatment

p300 has been shown to interact with multiple nuclear transcription factors, such as AP-1, CREB, hypoxia-inducible factor-1 and Elk1, as well as members of the core RNA transcriptional machinery, suggesting a role as a transcriptional integrator or adapter. After determining that DDT signals these same transcription factors potentially through a more general mechanism, we examined whether DDT and its metabolites could stimulate the coactivator p300. HEK 293 cells exposed to \( \text{o.p'-'DDT, p.p'-'DDT or o.p'-'dichlorophenyl dichloroethane} \) showed a potentiation of GAL4-fusion protein of full-length p300, but not the empty GAL4 expression vector (Figure 3A). Therefore, we can conclude that p300 contains a DDT-regulated transcriptional activation function.

The p38 MAPK signaling cascade activates p300

Since DDT-induced AP-1 activity is mediated through p38 MAPK, we tested if p38 MAPK affected p300 activity. CA-MKK mutants transfected into HEK 293 cells in conjunction with the GAL4-coactivator construct showed that CA-MKK6, which selectively activates p38, was the only M KK that significantly stimulated p300 (Figure 3B).

To demonstrate that DDT-induced coactivator activity is mediated through p38, we blocked \( \text{o.p'-'DDT-stimulated coactivator activity} \) with both molecular and pharmacological inhibitors of the various MAPK pathways. HEK 293 cells were transfected with increasing amounts of DN-ERK2, JNK1 or p38\( \alpha \) along with a GAL4 reporter plasmid and a full-length GAL4-p300 chimera followed by treatment with vehicle (DMSO) (Figure 4A) or 50 \( \mu \text{M} \) \( \text{o.p'-'DDT} \) (Figure 4B). DN-p38\( \alpha \), but not DN-ERK2 or DN-JNK1, inhibited DDT-induced p300 activity (Figure 4B). All inhibitory effects were significantly greater (\( P < 0.05 \)) in p38\( \alpha \)-DDT-treated cells versus vehicle-treated cells. DN-p38\( \alpha \) significantly inhibited (\( P < 0.001 \)) o.p'-'DDT-induced p300 activity (70–80\% inhibition). Additionally, DN-ERK2 also inhibited p300, albeit to a lesser degree than DN-p38\( \alpha \).

To confirm our molecular inhibitory findings, we blocked DDT-induced coactivator activity with pharmacological inhibitors of the MAPK pathways. GAL4 empty expression vectors or GAL4-coactivator fusions were transfected into HEK 293 cells and treated with vehicle or different MAPK inhibitors for 1 h, followed by treatment with vehicle or 50 \( \mu \text{M} \) o.p'-'DDT. The p38 pharmacological inhibitor SB203580 significantly blocked (\( P < 0.01 \)) o.p'-'DDT induction of p300, whereas there was no effect on cells transfected with empty GAL4 expression plasmid (Figure 4C). The ERK inhibitor, UO126, significantly inhibited (\( P < 0.05 \)) p300. The p300 inhibition by UO126 was not as potent as the effects caused by SB203580. Collectively, these data confirm that the transcriptional coactivator p300 is activated by DDT via the p38 MAPK pathway.

p38 phosphorylates p300

Recent reports have demonstrated that various kinases, including the MAPKs, ERK and JNK, can potentiate p300 through phosphorylation (11–13, 25–28). We hypothesized that p38 MAPK phosphorylates p300, leading to its potentiation. To test this, we bacterially expressed recombinant p300 using GST purification. Western blots using antibodies against GST revealed that GST-fusion proteins broke down into smaller fragments (data not shown), consistent with what others have seen (13). A map of the GST-fusion proteins tested is shown (Figure 5A). Purified fusion proteins were then subjected to an in vitro kinase assay in the presence of \( ^{32}\text{P} \) and activated p38 MAPK. The p300 N-terminal fragment containing amino acids 1–596 was the only p300 fragment phosphorylated (Figure 5B). These data demonstrate for the first time that p38 MAPK directly phosphorylates and activates p300.

Identification of domains of p300 involved in p38 responsiveness

To determine the specific regions p38 potentiates p300 and to complement the in vitro kinase data, HEK 293 cells were transfected as before with either empty expression vector or CA-MKK6 for 6 h in conjunction with GAL4-coactivator fragment constructs. A map of the GAL4-protein fusions is shown (Figure 6A). Cells were then treated with vehicle or \( \text{o.p'-'DDT (50 \( \mu \text{M} \))} \) overnight. Hence, p38 was stimulated both by molecular (CA-MKK6) and pharmacological (o.p'-'DDT) means. Consistent with our in vitro kinase data, both CA-MKK6 and o.p'-'DDT potentiated the N-terminus of p300 (Figure 6B). Specifically, o.p'-'DDT significantly increased (\( P < 0.001 \)) both fragments containing amino acids 1–596 and 1–743. CA-MKK6, however, only significantly increased (\( P < 0.05 \)) the p300 fragment containing amino acids 1–743, indicating that the major p38 target site is located in a region we were unable to detect in our in vitro kinase assay as the N-terminal GST-p300 fusion protein was missing amino acids 597–743. Thus, our in vitro kinase data.

Fig. 3. DDT and its metabolites stimulate the coactivator p300 via the p38 pathway. (A) HEK 293 cells were cotransfected for 6 h with 25 ng empty GAL4 expression plasmid or GAL4-p300 full length, along with a GAL4-luciferase reporter (50 ng) followed by overnight treatment as indicated in figure. The following day, luciferase activity was assayed. Results describe the fold induction over vehicle \( \pm \text{SE (n = 4)} \). *Indicates statistical significance (\( P < 0.05 \), ** \( P < 0.001 \)). (B) HEK 293 cells were cotransfected overnight with 25 ng of GAL4-p300 full length along with a GAL4-luciferase reporter (50 ng) and 100 ng of empty expression vector or CA-MKK1, 5, 6 or 7. All CA-MKK vectors were driven by a cytomegalovirus promoter. The following day, luciferase activity was assayed. Results describe the fold induction over vehicle \( \pm \text{SE (n = 4)} \). *Indicates statistical significance (\( P < 0.05 \), ** ** \( P < 0.001 \)).
strongly correlate with the GAL4 studies and shows that p38 targets the N-terminus of p300.

**Discussion**

Coactivators are critical components involved in the process of transcriptional initiation and elongation. The general coactivator p300 is essential to basically all cellular functions, including growth, differentiation and apoptosis (4). Thus, determining the regulation of this coactivator is central to understanding how signaling mechanisms control gene expression and ultimately biological function. Here, we demonstrate that the p38 MAPK phosphorylates and potentiates the nuclear coactivator p300.

The pesticide DDT was previously demonstrated by us to signal p38, leading to increased AP-1-mediated gene expression (18,19). The increased AP-1 activity was due to (i) increased expression of c-jun and (ii) post-translational activation of c-Jun and c-Fos. We show here that p38 activation leads to increased transcription of Fra-1, a component of the AP-1 complex. Therefore, the DDT-stimulated AP-1 activity could be due to a third factor, a p38-induced increase in Fra-1 expression. The data presented here indicate that DDT-induced activity also increases the transcriptional activities of other transcription factors such as CREB and Elk1. In support of this, we show that DDT increases gene expression through cyclic adenosine monophosphate and hypoxia-responsive elements (Figure 2A and B). While p38 has been shown to target Elk1, CREB, hypoxia-inducible factor-1 and the c-jun promoter (1,28,29), how it potentiates c-Jun and c-Fos is unclear (18,30). All these transcription factors recruit common coactivators, indicating that perhaps p38 signaling to the transcription factors could be accomplished by an indirect potentiation, one favoring the shared coactivators. Mammalian one-hybrid assays using different GAL4-coactivator constructs demonstrate that o,p'-DDT, p,p'-DDT and o,p'-DDE, which were shown previously to stimulate AP-1 activity (19), stimulated p300 (Figure 3A). Another DDT metabolite found in humans, p,p'-dichlorodiphenyl acetic acid, which does not potentiate AP-1, likewise had no effect on p300. While DDT was used primarily as a pharmacological tool to stimulate p38 activity, these data do also represent a novel mechanism by which environmental compounds can co-opt transcriptional regulation.

Since DDT induces AP-1 activity through p38, we sought to determine the effect of MAPK signaling on p300 using both pharmacological and molecular regulators of the ERK, JNK and p38-signaling pathways. CA-MKK6 significantly increased p300 transcriptional activity (Figure 3B). Conversely, both a DN mutant of p38 and SB203580, a pharmacological inhibitor of p38, blocked DDT-induced coactivator signaling (Figure 4). This potentiation also appeared to be due, in part, to the ERK signaling cascade, although to a lower degree. ERK has previously been demonstrated to potentiate p300...
However, it must be noted that CA-MKK1 did not stimulate p300 in HEK 293 cells in our hands, contrasting the UO126 and DN-ERK data as well as the effects seen by other labs using HeLa cells and cardiac myocytes. Obviously, additional work is required to understand the exact role of the ERK signaling cascade in p300 potentiation in HEK 293 cells before conclusions can be drawn. Our results shown here mirror the effect DDT has on AP-1-mediated transcription, indicating those results were most probably not due to targeting of AP-1 itself, but rather a targeting of the AP-1-associated proteins.

Sequence analysis indicates several possible p38 MAPK sites in p300, and we demonstrate in vitro that activated p38 phosphorylates p300 (Figure 5B). Consistent with what other labs have shown for the ERK MAPK signaling cascade, p38 phosphorylates the N-terminal region of p300 (10–12). However, Sang et al. (12) did demonstrate that ERK phosphorylates the C-terminus of p300 in HeLa cells, demonstrating the cell-specific effects kinase signaling pathways can have on protein substrates. GAL4 one-hybrid assays confirm the same regions we demonstrated to be phosphorylated in vitro are targeted in vivo (Figure 6B). While p38 phosphorylated a p300 fragment containing amino acids 1–596, this phosphorylation neither appeared very strong nor did CA-MKK6 significantly potentiate the corresponding GAL4 fusion. The potentiation seen with the pharmacological stimulant may have been caused by another kinase stimulated by o,p’-DDT, such as ERK, as sequence analysis suggests that S133 and T524 are potential proline-directed kinase sites. However, no one has identified if these sites are targeted. CA-MKK6 did, however, significantly stimulate the GAL4-p300 fragment containing amino acids 1–743, indicating that an unidentified p38-responsive site lies between amino acids 596 and 743 (Figure 6). Unfortunately, our in vitro kinase assay did not address this small but apparently important region. This region contains part of the KIX domain, which binds certain transcription factors such as CREB, c-Jun, activating transcription factor-1/2 and c-myc as well as the TATA-binding protein (3). It would be interesting to see if targeting of this section of p300 alters binding to transcription factors or even components of the basal transcription machinery. It must be noted, however, that the C-terminal p300 fragment (amino acids 1572–2414) was difficult to purify in high concentrations as seen by the light coomassie blue-stained bands in Figure 5B. It still is possible that with greater amounts of this protein fragment present, there may be some phosphorylation. However, our results here detecting no signal combined with p38’s inability to potentiate this region (Figure 6B) in the mammalian one-hybrid assay suggest that this is not the case.

Our results demonstrate a novel mechanism for p38-induced transcription. Transcription factors such as Elk1, activating transcription factor-2 and ER are all convergent points for p38-signaling pathways. Some transcription factors such as Elk1 and activating transcription factor-2 are directly phosphorylated by p38. Here, we show another level of p38-transcriptional regulation, p38 targeting of the recruited coactivator p300. This added requirement may help p38 selectively activate target genes.

**Funding**

Department of Energy (DE-FC26-00NT40843 to J.A.M.); Office of Naval Research (N00014-99-1-0763 to J.A.M. and M.E.B.); Center for Disease Control (RO6/CCR419466-02 to J.A.M.); National...
Institutes of Health (DK059389 to M.E.B.); Cancer Association of Greater New Orleans (to D.E.F.); Louisiana Cancer Research Consortium (to M.R.B.); Susan G. Komen Breast Cancer Foundation (BCTR0601198 to M.E.B.).

Acknowledgements

Conflict of Interest Statement: None declared.

References


Fig. 6. Effect of CA-MKK6 or o,p′-DDT on the transcriptional activity of different p300 domains per mutants. (A) Schematic of GAL4-fusion protein constructs used for mammalian one-hybrid assays. (B) HEK 293 cells were cotransfected for 6 h with 25 ng of GAL4 empty expression vector or different GAL4-p300 full-length or mutant vectors as indicated in the figures along with a GAL4-luciferase reporter (50 ng). Hashed bars represent cells that were also transfected overnight with 100 ng of empty expression vector or CA-MKK6. Black bars represent cells that were treated overnight with either vehicle or o,p′-DDT (50 μM). The following day, luciferase activity was assayed. Results describe the fold stimulation over vehicle-transfected cells (black bars) or vehicle-treated cells (hashed bars) ±SE (n = 4). * Indicates statistical significance (*P < 0.05, **P < 0.01, ***P < 0.001).


Received July 21, 2008; revised August 28, 2008; accepted September 1, 2008