cFos Represses Transcriptional Activation by the Ah Receptor : Cross-Talk between Signal Transduction Pathways

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Abstract

The effects of expressing cFos in mouse hepatoma Hepa 1c1c7 cells were measured using a TCDD-inducible CAT reporter gene and sucrose density gradient ananysis of nuclear Ah receptor. In cells transfected with reporter plasmid alone, TCDD caused a 3.2 fold increase in CAT activity compared to solvent-treated control cells. The CAT activities in cells cotransfected with the TCDD-inducible plasmid and 5, 10 and 20μg of the cFos expression plasmid were 1.5-, 0.8- and 0.2-fold respectively, compared to the control cells. Furthermore, a higher molecular weight nuclear Ah receptor (10.9 S) complex was formed in cells expressing cFos compared to controll cells and evidence for direct protein-protein interactions was supported by immunoprecipitation of nuclear [^{3}H]-TCDD-Ah receptor with antibodies to cFos. These results are consistent with the formation of a cFos-Ah receptor complex which resulted in the inhibition of TCDD-induced gene transcription thus suggesting a new mechanistic model for the multiple interactions and cross-talk between the Ah receptor and other endocrine response pathways.

Introduction

The intracellular aryl hydrocarbon (Ah) receptor is a unique ligand-activated transcriptin factor (LTF) which, in its liganded state, transactivates several target genes including those encoding CYPIA1, the glutathione S-transferase Ya subunit, and NAD(P)H:quinone reductase[1]. The transcription factor AP-1 is composed of two subunits, cFos and cJun, and responds to a number of physiologic (IL-1, PDGF, estrogen) and unphysiologic agents (ultraviolet radiation, tumor promoting agents)[2]. Experimental evidence indicates that cross-talk between the AP-1 proteins and *several LTF's occurs* with resultant effects on gene regulation [3]. Recently, several inducers of AP-1 have been shown to inhibit TCDD-induced CYP1A1 mRNA. For

example, TPA inhibited the induction of CYP1A1 and CTP1A2 gene expression by TCDD and related compounds in C57BL/6 mice and the induction of CYP1A1 gene expression in Hepa-1c1c7 cells [4-5]. In a separate study IL-1β was shown to inhibit TCDD induced CYP1A1 and CYP1A2 mRNA in isolated rat hepatocytes [6]. This study demonstrates that cFos oncoprotein can mimic the inhibitory effects of AP-1 inducers on the transcriptional activating capacity and molecular properties of the Ah receptor in Hepa1c1c7 cells.

MATERIALS AND METHODS

Chemicals and Biochemicals: All chemicals and cell culture media and serum were purchased from Sigma Chemical Company, St. Louis, MO. unless otherwise specified. Plastic cell culture supplies were purchased from Corning Science Products, Corning, N.Y. 2,3,7,8-Tetrachlorodibenzofuran (TCDF) unlabeled TCDD and [^{3}H]TCDD (37 Ci/mmol) were synthesized in this laboratory[7].

Cell Culture: Hepa 1c1c7 cells were kindly provided by Dr. J. P. Whitlock, Jr., Stanford University and were cultured as previously described [7].

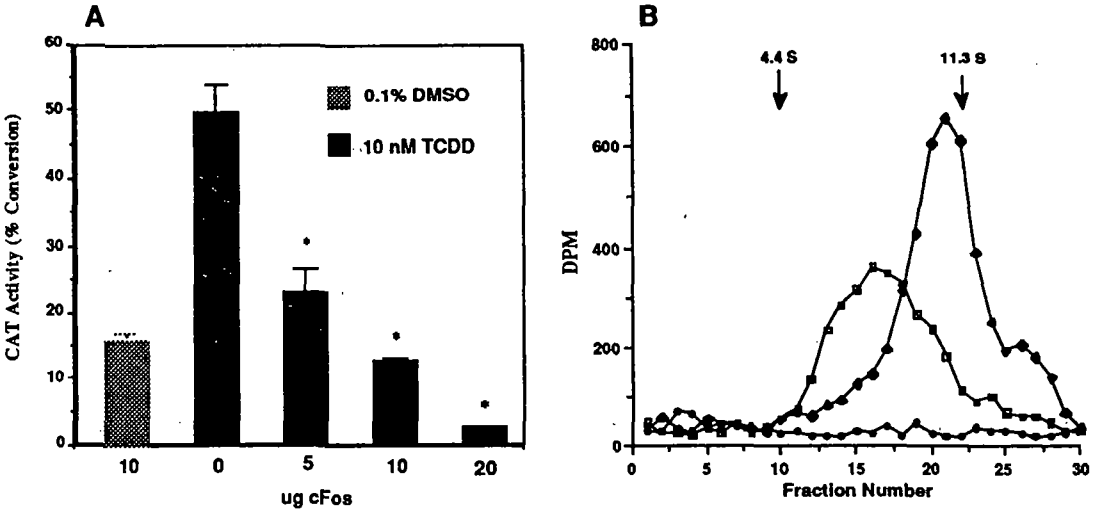
Sucrose Density Gradient Analysis of Nuclear Extracts: Hepa-1 cells in 150 cm^2 flasks were transfected with the plasmids as described below. Nuclear extracts from Hepa 1c1c7 cells which were treated for 1 hr with 10 nM [^{3}H]TCDD in the presence or absence of 5 μM unlabeled TCDF were prepared as previously described [9]. Aliquots (150 μl) of liganded nuclear extracts were layered onto linear sucrose gradients (10-30%) prepared in HEG buffer (25 nM HEPES, 1.5 mM EDTA, 10% glycerol, pH 7.6). Gradients were centrifuged at 4°C for 3 hr at 404,000 x *g* and subsequently separated into 30 fractions as described [7]. The radioactivity in each fraction was determined by liquid scintillation counting and corrected for counting efficiency. The concentration of Ah receptor was determined from the amount of specifically-bound radiolabeled receptor complex [7].

Cell Transfections and CAT Assays: DNA transfections and CAT assays were essentially carried out as described[8]. Cells were transfected with 10 μg of pMCAT 5.12 or coprecipitated with varying amounts of pUCSV-mfos and the total amount of DNA was adjusted to 30 μg/dish with pSV2Apap. Twenty-four hr after the glycerol shock, TCDD, when used, was added to a final concentration of 10^{-8} M, and cells were incubated a further 24 hr. Cells were harvested by scraping from the plate and protein concentrations were determined by the Bradford method[8]. CAT activity was measured from samples incubated for 90 min at 37°C. The resulting products were quantitated on a Betascope 603 imaging system (Betagen, Inc.) and the percent conversion from unacetylated to acetylated [^{14}C]-chloramphenicol was determined. The mouse genomic c-*fos* expression vector pUCSV-mfos was a gift from Dr. Michael Karin, La Jolla, CA, and has been described elsewhere[9]; the DRE-MMTV-CAT construct (pMCAT 5.12) was a gift from Dr. J. P. Whitlock, Jr., Stanford, CA; pSV2Apap was a gift from Dr. Tom Kadesch, Philadelphia, PA[10].

Immunoprecipitation: Hepa 1c1c7 cells were transfected with pUCSV-mfos for 24 hr and treated with 10 nM [^{3}H]TCDD for 2 hr. Nuclear extracts were prepared as previously described[8] and immunoprecipitations were carried out as described[11]. Nuclear extracts (100 μg) were precipitated with anti-cFos serum (anti-b-gal-fos fusion protein corresponding to amino acids 151-292 of mouse cFos, Medac

Diagnostica, Hamburg, Germany) The radioactivity in each precipitate was determined by liquid scintillation counting and the data are expressed as net dpm/100 µg protein, where net dpm is (dpm sample) - (dpm background).

Results and Discussion



* -Significantly lower ($p < 0.01$) then cells treated with TCDD alone.

Figure 1; **A** *Effects of 10 nM TCDD and different amounts of pUCSV-mfos plasmid* on pMCAT 5.12 activity in Hepa 1c1c7 cells. All the cells were transfected with 10 µg pMCAT 5.12 and the indicated amount of pUCSV-mfos plasmid and treated with either 10 nM TCDD or 0.1% DMSO. The data is from triplicate samples expressed as the % conversion of [^{14}C]-Chloramphenicol to its acetylated form. **B** Velocity sedimentation analysis of nuclear extracts from Hepa 1c1c7 cells treated with 10 nM [^{3}H]TCDD. The nuclear extracts were obtained from cells treated with 10 nM [^{3}H]TCDD (- □ -), 10 nM [^{3}H]TCDD plus 40 µg of the pUCSV-mfos plasmid (- ◆ -), and 10 nM [^{3}H]TCDD plus 2 µM TCDF (- ● -).

The results in Figure 1A summarize the concentration-dependent effects of transiently transfected cFos expression vector (pUCSV-mfos) on the CAT activity in Hepa 1c1c7 cells cotransfected with pMCAT 5.12. The pMCAT 5.12 plasmid construct is composed of TCDD-inducible heterologous promoter containing a genomic DRE (DRE-3) derived from the 5'-flanking region of the CYP1A1 gene and an MMTV promoter fused to a bacterial CAT reporter gene. In cells cotransfected with the pMCAT 5.12 and pUCSV-mfos (10 µg) plasmids in the presence or absence of 10 nM TCDD, the relative percent CAT activity was 13.0 ± 0.4% and 15.6 ± 1.3%, respectively. These results indicated that the activity of TCDD as an inducer of CAT activity was suppressed in the presence of cFos. In contrast, in cells treated with 10 nM TCDD, pMCAT 5.12 and no pUCSV-mfos, there was a significant induction of CAT activity (50.1 ± 4.0% of conversion) and this induced response was decreased with increasing amounts of cotransfected pUCSV-mfos plasmid (Figure 1A).

In a separate experiment, Hepa 1c1c7 cells were treated with 10 nM [^{3}H]TCDD, 10 nM [^{3}H]TCDD plus 2 μM TCDF or 10 nM [^{3}H]TCDD plus 40 μg of pUCSV-mfos plasmid. The levels of specifically-bound nuclear [^{3}H]TCDD Ah receptor complex in the cells treated with 10 nM [^{3}H]TCDD alone and 10 nM [^{3}H]TCDD plus 40 μg of the pUCSV-mfos plasmid were 183 ± 13 fmol/mg protein and 275 ± 18 fmol/mg protein, respectively. Velocity sedimentation analysis of the radiolabeled nuclear extracts from the Hepa 1c1c7 cells is illustrated in Figure 1B. The nuclear Ah receptor complex from cells treated with 10 nM [^{3}H]TCDD alone sedimented at 8.2 ± 0.34 S whereas in Hepa 1c1c7 cells treated with [^{3}H]TCDD plus 40 μg of the pUCSV-mfos plasmid the liganded complex sedimented at 10.9 ± 0.33 S. The possible complexation of cFos with the liganded nuclear Ah receptor complex is further supported by immuno-precipitation of these nuclear extracts with cFos antibodies. The net dpm/100 μg nuclear protein in nuclear extracts from Hepa 1c1c7 cells treated with (i) 10 nM [^{3}H]TCDD, 2 μM TCDF plus cFos antibodies, (ii) 10 nM [^{3}H]TCDD plus 40 μg pUCSV-mfos plasmid, and (iii) 10 nM [^{3}H]TCDD alone was 3.9 ± 2.0, 16.6 ± 4.0 and 9.7 ± 1.9 dpm/100 μg nuclear protein, respectively.

The results of this study demonstrate that the Ah receptor-mediated signal transduction pathway can be modulated by an interaction between the nuclear Ah receptor complex and cFos, a member of the AP-1 subfamily of bZIP nuclear factors. Cross-coupling between AP-1 and intracellular LTF's has been described for several hormone receptors[3]. This study extends the growing list of nuclear protein-protein interactions which can modulate gene expression and suggests a new model which may be useful for describing the myriad of interactions between the Ah receptor and other endocrine response pathways .

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