Chromium(VI) Regulation of Gene Expression Has Been Attributed to the Generation of Reactive Chromium and Oxygen Species, DNA Damage, and Alterations in mRNA Stability. However, the Effects of Cr(VI) on Signal Transduction Leading to Gene Expression Are Not Resolved. Therefore, This Study Investigated the Effects of Cr(VI) on Basal and Tumor Necrosis Factor-α (TNF-α)-Induced Transcriptional Competence of Nuclear Factor-κB (NF-κB) in A549 Human Lung Carcinoma Cells. Pretreatment of A549 Cells with Nontoxic Levels of Cr(VI) Inhibited TNF-α-Stimulated Expression of the Endogenous Gene for Interleukin-8 and of an NF-κB-Driven Luciferase Gene Construct, But Not Expression of Urokinase, a Gene with a More Complex Promoter. Chromium Did Not Inhibit TNF-α-Stimulated IkBo Degradation or Translocation of NF-κB-Binding Proteins to the Nucleus. However, Cr(VI) Pretreatments Prevented TNF-α-Stimulated Interactions Between the p65 Subunit of NF-κB and the Transcriptional Coactivator cAMP-Responsive Element-Binding Protein-Binding Protein (CBP). This Inhibition Was Not the Result of an Effect of Chromium on the Protein Kinase A Catalytic Activity Required for p65/CBP Interactions. In Contrast, Cr(VI) Caused Concentration-Dependent Increases in c-Jun/CBP Interactions. These Data Indicate That Nontoxic Levels of Hexavalent Chromium Selectively Inhibit NF-κB Transcriptional Competence by Inhibiting Interactions with Coactivators of Transcription Rather Than DNA Binding.

Chromium(VI) Promotes Pulmonary Fibrosis and Is a Human Carcinogen. Cr(VI) Affects Expression of Various Genes, Including Catalase, Heme Oxygenase, 5-Aminolevulinate Synthase, and Urokinase Plasminogen Activator Receptor (1–3). These Chromium-Mediated Changes in Gene Expression Are Attributed to the Generation of Reactive Chromium and Oxygen Species, DNA Damage, or Alterations in mRNA Stability. This Study Examined Whether Cr(VI) Has Additional Effects on Gene Expression at the Level of Transcription Factor Activation or Transcriptional Competence. This Type of Epigenetic Interference with the Transcriptional Machinery Would Have Profound Effects by Shifting the Patterns of Genes Expressed Without Causing Molecular Damage. Nuclear Factor-κB (NF-κB) Is a Mammalian Transcription Activator Protein Involved in Regulating Expression of Immune and Inflammatory Response Genes. It Occurs in Both Homo- and Heterodimeric Forms. The Most Common Transcriptionally Competent Form Is Composed of a p50 DNA-Binding Subunit Attached to a p65 Transactivation Subunit. In Nonstimulated Cells, NF-κB Is Localized in the Cytoplasm Bound to Its Inhibitor, IκB, and to the Catalytic Subunit of Protein Kinase A (PKA) (4). Upon Cellular Activation by Cytokines, Viral Infection, Lipopolysaccharide, and Reactive Oxygen Species, IκB Is Phosphorylated and Degraded. This Degradation Unmasks the ATP-Binding Site on PKA, Resulting in Activation and NF-κB p65 Phosphorylation (4). Furthermore, Loss of IκB Exposes the NF-κB Nuclear Localization Sequence, Allowing NF-κB to Translocate to the Nucleus and to Bind to Its Consensus Sequence Within the Promoter Region of Genes.

Studies Have Shown That, in Addition to DNA Binding, the Interaction of p65 with CREB-Binding Protein (CBP) Is Essential for NF-κB-Enhanced Transcriptional Activity (5, 6). CBP Is a Coactivator Molecule That Links Enhancer-Bound Transcription Factors (Transcription Factor IIB and TATA-Binding Protein) to the Basal Transcriptional Machinery. Interaction of CBP with p65 Occurs at Two Sites. PKA-Induced Phosphorylation of p65 on Serine 276 Mediates the Phosphorylation-Dependent Interaction of p65 with the KIX Region of CBP (Amino Acids 452–661). Alternatively, the C-Terminal Portion of p65 Interacts with CBP in a Phosphorylation-Independent Manner (5). In Addition to p65, CBP Interacts with Various Other Transcription Factors, Including CREB, c-Jun, c-Fos, p53, Glucocorticoid Receptor, and Retinoic X Receptor (7–11). Thus, Differences in the Interaction of p65 with CBP May Occur as a Result of Decreases in p65 Phosphorylation or Competition Between Transcription Factors for Limiting Quantities of CBP.

In This Study, the Complex Multistep Pathway for NF-κB-Dependent Gene Expression Was Chosen to Examine How Nontoxic Concentrations of Cr(VI) Produce Epigenetic Effects on Cell Phenotype. The Effects of Cr(VI) on Both Basal and TNF-α-Stimulated Gene Expression Were Investigated in A549 Human Lung Carcinoma Cells. Cr(VI) Inhibited Both Basal and Stimulated Expression of an NF-κB-Driven Luciferase Reporter Construct and the Endogenous Gene for IL-8. Since There Was No Effect of Cr(VI) on the Pathways That Signal for Translocation of NF-κB

*This work was supported by Environmental Protection Agency Superfund Basic Research Program Project Grant ES07373 and NHLBI, National Institutes of Health Grant HL52738. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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1 The abbreviations used are: NF-κB, nuclear factor-κB; PKA, protein kinase A; PKAc, protein kinase A catalytic subunit; CREB, cAMP-responsive element-binding protein; CBP, cAMP-responsive element-binding protein-binding protein; TNF-α, tumor necrosis factor-α; IL, interleukin; PCR, polymerase chain reaction; uPA, urokinase-type plasminogen activator; PVDF, polyvinylidene difluoride; PAGE, polyacrylamide gel electrophoresis.
to the nucleus or its DNA binding, the interaction of p65 with CBP was examined. The data obtained indicate that Cr(VI) selectively inhibited this interaction, whereas it increased interactions between CBP and other transcription factors such as c-Jun. Thus, hexavalent chromium selectively disrupts gene expression by altering interactions between specific transcription factors and their cofactors.

MATERIALS AND METHODS

Cell Culture—Unless otherwise stated, A549 cells (American Type Culture Collection, Manassas, VA) were grown to 100% confluence at 37 °C and 5% CO2 using nutrient medium F-10 supplemented with 10% fetal bovine serum, 60 μg/ml penicillin, and 100 μg/ml streptomycin. The medium was changed on 1-day post-confluent cells prior to treatment.

RNA Isolation and Reverse Transcription-PCR—Total cellular RNA was isolated using Trizol reagent (Life Technologies, Inc.) according to the manufacturer’s instructions. Reverse transcription-PCR was performed on 0.5 μg of the resulting RNA pellet according to previously published methods (3). IL-8 primers (forward, 5′-atacgctctcaaacaatactc-3′; and reverse, 5′-tcgctgctgctgagct-3′), human uPA primers (forward, 5′-aaaatgctgtgtgctgctgacc-3′; and reverse, 5′-gggcgatgatcttgatcttc-3′), and β-actin primers (forward, 5′-ggagctgcccctc-3′; and reverse, 5′-ggcggatgattatgc-3′) were all synthesized in the Dartmouth Molecular Core facility.

Transfections—Cells were plated at 1 × 10^6 cells/well and transfected at 70–80% confluence according to the LipofectAMINE Plus protocol (Life Technologies, Inc.). Briefly, 1.5 μg of p55Igκ construct containing three NF-κB repeats placed in front of the minimal interferon-β promoter (−55 to +19) driving the luciferase gene (12) and 0.5 μg of BioGreen pRK5 mammalian expression vector expressing green fluorescent protein (Pharmingen, San Diego, CA) were incubated with Plus reagent for 15 min in serum- and antibiotic-free medium. LipofectAMINE was then added, and the mixture was incubated for an additional 6 h. The transfection reagent remained on the cells for 3 h. Following incubation, the medium was changed to antibiotic-free medium containing 10% fetal bovine serum, and cells were treated 36 h later for 2 or 4 h. The medium was changed after treatment, and luciferase assays were performed 8 h later.

Luciferase Assay—Cells were lysed using 250 μl of lysis buffer (25 mM glycyglycine, 4 mM EGTA, 15 mM MgSO4, 1% Triton X-100, and 1 mM dithiothreitol), and samples were centrifuged. Supernatants (50 μg) were combined with 150 μl of assay buffer (25 mM glycyglycine, 15 mM potassium phosphate, 15 mM MgSO4, 4 mM EGTA, 2 mM ATP, and 1 mM dithiothreitol), and relative light units were determined using a luminometer upon addition of 400 μM d-luciferin potassium (Analytical Luminescence Laboratory, San Diego, CA). Total nuclear proteins from supernatants were quantified by Coomassie Plus assay (Pierce) using bovine serum albumin as a protein standard.

Gel Mobility Shift Assays—Gel mobility shift assays were performed as described previously (13, 14). Total nuclear proteins were isolated from chromium-treated cells and quantified as described above. Typical sample, 5 fmol of 5′-32P-labeled double-stranded oligonucleotide containing the consensus binding sequence for NF-κB (5′-ccggctgagctgctggagg-3′; and reverse, 5′-cccgccct-3′) or a 7.5% (CBP immunoprecipitate) or 10% (p65 immunoprecipitate) SDS-polyacrylamide gel and transferred to PVDF membranes (Millipore Corp., Bedford, MA). Membranes were immunoblotted with affinity-purified polyclonal antibody to p65, CBP, or c-Jun or with monoclonal antibody to PKAc for 60 min in TWEEN-20/Tris-buffered saline contain-

FIG. 1. Effects of Cr(VI) on TNF-α-stimulated IL-8 mRNA levels. A, 1-day post-confluent cells were cotreated for 2 h with 5 or 20 μM Cr(VI) and 10 ng/ml TNF-α. B, cells were pretreated with 1, 5, or 20 μM Cr(VI) for 2 h prior to addition of 10 ng/ml TNF-α for an additional 2 h. Total RNA was isolated at the end of the treatment period. IL-8 and β-actin mRNAs were then measured following reverse transcription-PCR with gene-specific primers. DNA was amplified through 20 (β-actin) or 21 (IL-8) rounds of PCR.

RESULTS

Cr(VI) Decreases TNF-α-stimulated IL-8 mRNA Expression—IL-8 mRNA is not expressed basally in A549 cells, and previous work has demonstrated that it is not induced in response to Cr(VI) (15). To determine the effects of Cr(VI) on induction of IL-8 mRNA, cells were either untreated for 2 h with Cr(VI) and TNF-α or pretreated with Cr(VI) for 2 h prior to addition of TNF-α for an additional 2 h. Cotreatment with 5 or 20 μM Cr(VI) had no effect on IL-8 mRNA levels (Fig. 1A). Alternatively, pretreatment for 2 h with concentrations of Cr(VI) as low as 5 μM significantly decreased TNF-α-stimulated IL-8 mRNA levels (Fig. 1B). To determine whether the decrease in mRNA levels was specific for IL-8, uPA mRNA levels were examined after pretreatment of the cells with chromium followed by treatment with TNF-α. In contrast to IL-8 expression, pretreatment with 5 μM Cr(VI) had no effect on TNF-α-stimulated uPA mRNA levels. Nevertheless, pretreatment with 20 μM Cr(VI), which is toxic in this model (3, 16), significantly decreased uPA mRNA levels (Fig. 2).

Cr(VI) Decreases NF-κB Transcriptional Activity—To determine if the decrease in IL-8 mRNA levels was due to an effect on NF-κB activity, cells were transiently transfected with plas-
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Fig. 2. Effects of Cr(VI) on TNF-α-stimulated uPA mRNA levels. A549 cells were incubated in the absence or presence of 1, 5, or 20 μM Cr(VI) for 2 h prior to addition of buffer or 10 ng/ml TNF-α. The cells were incubated for an additional 2 h before isolation of total RNA. After reverse transcription, DNA was amplified through 21 or 23 rounds of PCR with primers specific for β-actin or uPA cDNA, respectively. Data represent triplicate experiments.

mids containing an NF-κB enhancer region upstream of the interferon-β minimal promoter driving expression of the luciferase gene. Transfected cells were treated for 2 h with 1, 5, or 20 μM Cr(VI) or cotreated with the varying concentrations of Cr(VI) and 10 ng/ml TNF-α for 2 h. Treatment of cells with up to 20 μM Cr(VI) alone for 2 h had no effect on luciferase levels, whereas treatment with TNF-α increased luciferase levels 2-fold above control levels (Fig. 3A). Cotreatment with TNF-α and 5 or 20 μM Cr(VI) resulted in reduction of luciferase levels to 50% as compared with TNF-α-stimulated levels. To confirm equivalent transfection efficiency, a pRK5 plasmid expressing fluorescent green protein was cotransfected into the cells. No differences in transfection efficiency were observed for all treatment groups (data not shown).

In contrast to the 2-h treatments with Cr(VI), a 4-h treatment with 5 and 20 μM Cr(VI) decreased luciferase levels in a dose-dependent manner (Fig. 3B). Likewise, pretreatment with 5 and 20 μM Cr(VI) prior to addition of TNF-α decreased luciferase levels by 50 and 75%, respectively, as compared with TNF-α-stimulated luciferase levels alone.

To demonstrate that the concentrations of Cr(VI) used to treat the cells had no effect on luciferase enzyme activity or did not significantly impair the cellular transcriptional machinery, cells were transfected with a pGL3 plasmid containing an SV40 enhancer and promoter and were treated with varying concentrations of Cr(VI). Consistent with previous demonstrations of toxicity in this model (3, 16), 20 μM Cr(VI) decreased luciferase expression as compared with untreated controls or cells treated with 10 μM Cr(VI) or less (Fig. 3C). Decreased expression may be caused by either DNA damage or inhibition of protein synthesis (3, 16).

Cr(VI) Has No Effect on NF-κB Translocation and DNA Binding—To determine if a lack of NF-κB nuclear translocation or DNA binding was responsible for the decrease in transcriptional activity, cells were treated with 5, 10, or 20 μM Cr(VI) for 1, 2, or 4 h, and nuclear proteins were analyzed using gel mobility shift assay. All concentrations of Cr(VI) up to 20 μM had no effect on NF-κB nuclear translocation or binding to its DNA consensus sequence (Fig. 4A). Similarly, cotreatment or pretreatment with 20 μM Cr(VI) had no effect on TNF-α-stimulated NF-κB nuclear translocation (Fig. 4B). Finally, the effect of chromium on TNF-α-induced degradation of IκB was examined to demonstrate whether chromium affected the signal transduction leading to IκB degradation or the degradation process itself. Chromium did not affect IκB protein levels under basal conditions (Fig. 5B). Pretreatment with chromium also had no effect on TNF-α-stimulated IκB degradation. These data indicate that Cr(VI) inhibits NF-κB-dependent gene expression at the level of preventing transcriptional competence, not at the level of translocation or DNA binding.

Fig. 3. Effects of Cr(VI) on NF-κB-dependent transcription. Cells were transiently transfected with p55IκBα as described under “Materials and Methods.” A, cells were treated with 0, 1, 5, or 20 μM Cr(VI) for 2 h in the absence or presence of 10 ng/ml TNF-α. B, cells were pretreated with chromium for 2 h prior to addition of TNF-α and incubation for an additional 2 h. C, cells were transfected with a fully competent pGL3 plasmid and then treated with increasing concentrations of Cr(VI) for 4 h. At the end of the treatment periods, the medium was replaced, and the luciferase activities were measured 8 h later. All luciferase determinations were normalized to total cellular protein. Data represent the means ± S.D. of three experiments. *, p < 0.05; **, p < 0.01; ***, p < 0.001. Significant differences between groups of cells in the absence of TNF-α are with respect to the control. Significant differences between groups treated with TNF-α are with respect to TNF-α in the absence of chromium. RLU, relative light units.

Cr(VI) Inhibits the Interaction of p65 with CBP—Transcriptional competence of NF-κB requires interaction with CBP (7). To examine the effects of chromium on this interaction, cells were incubated with Cr(VI) alone or with TNF-α after a 2-h pretreatment with Cr(VI). Total cell lysates were immunoprecipitated with antibody to CBP, and then the resulting proteins were probed for the presence of p65. The data in Fig. 5 indicate that preincubating the cells with either 5 or 20 μM Cr(VI) prior to adding TNF-α significantly decreased stimulated p65/CBP interactions. No interaction between p65 with CBP was observed in the absence of TNF-α stimulation. It is interesting to
note that the lack of basal p65/CBP interactions correlates well with the lack of basal IL-8 expression shown in Fig. 1. This may explain why there is no basal IL-8 expression even though there are basal levels of NF-κB-binding proteins in the nucleus (Fig. 4).

**Cr(VI) Has No Effect on PKA Activity**—PKAc-mediated phosphorylation of the p65 subunit is required for interaction of p65 with CBP. p65-associated PKA activation is dependent upon IκB degradation (4), which was not affected by Cr(VI) (Fig. 5B). To determine if the decreased interaction of p65 with CBP was due to a Cr(VI)-induced decrease in PKA catalytic activity, cells were treated with TNF-α or pretreated with 5 and 20 μM Cr(VI) prior to treatment with TNF-α. Total cAMP-inducible PKA activity was not inhibited by any of the treatments examined (Fig. 6A). The form of PKA responsible for phosphorylating p65 directly interacts with the p65 subunit. To confirm that there were no effects on this interaction, p65 was immunoprecipitated and analyzed by Western analysis with primary antibody specific for PKAc. Chromium did not affect the interaction of p65 with PKAc (Fig. 6B).

**Cr(VI) Increases Interactions between c-Jun and CBP**—The effect of Cr(VI) on CBP interactions with c-Jun was investigated to determine whether disruption of p65/CBP interactions was a specific or a general inhibition of CBP binding activity. The experimental paradigm in Fig. 5 was repeated, and immunoprecipitated proteins were then prepared and incubated with cAMP in the presence of [γ-32P]ATP and 50 μM Kemptide or Kemptide + 1 μM inhibitor (protein kinase inhibitor amide). Data are presented as the means ± S.D. of triplicate cultures. B, cells were treated as described for A. Total cell lysates were immunoprecipitated (IP) with antibody to p65. The resulting proteins were resolved by 10% polyacrylamide gel electrophoresis and transferred to PVDF membranes. Western analysis was then performed with antibody specific for either p65 or PKAc, followed by incubation with horseradish peroxidase-conjugated secondary antibodies. Immunoreactivity was detected by enhanced chemiluminescence.

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![Fig. 4. Effects of Cr(VI) on NF-κB DNA binding. A, A549 cells were treated with increasing concentrations of Cr(VI) or 10 ng/ml TNF-α for 4 h. B, triplicate cultures of cells were treated in the absence or presence of Cr(VI) or TNF-α for 4 h. In addition, various combinations of pretreatments and cotreatments were conducted. The time given is the total time that the cells were treated with the individual agent during the 4-h incubation period. At the end of the treatment periods, nuclear proteins were then extracted, and NF-κB binding in 0.5 μg of extract was determined by electrophoretic mobility shift assay.

![Fig. 5. Effects of Cr(VI) on TNF-α-induced IκB degradation and p65/CBP interactions. A, cells were incubated in the absence or presence of Cr(VI) for 2 h prior to adding TNF-α to the indicated groups. After an additional 2 h, total protein lysates were prepared and immunoprecipitated (IP) with antibody specific for CBP. The immunoprecipitated proteins were then separated by PAGE, transferred to PVDF membranes, and immunoblotted (IB) for either CBP or p65. B, a portion of the total protein lysate was separated by PAGE, transferred to PVDF membranes, and then immunoblotted for IκB. Antibody-antigen complexes were detected by binding to secondary antibodies conjugated to horseradish peroxidase, followed by enhanced chemiluminescence.

![Fig. 6. Effects of Cr(VI) on PKAc activity and interaction with p65. A, cells were incubated in the presence or absence of Cr(VI) for 2 h. TNF-α was then added to the indicated groups. Cytosolic (upper panel) and nuclear (lower panel) extracts were then prepared and incubated with cAMP in the presence of [γ-32P]ATP and 50 μM Kemptide or Kemptide + 1 μM inhibitor (protein kinase inhibitor amide). Data are presented as the means ± S.D. of triplicate cultures. B, cells were treated as described for A. Total cell lysates were immunoprecipitated (IP) with antibody to p65. The resulting proteins were resolved by 10% polyacrylamide gel electrophoresis and transferred to PVDF membranes. Western analysis was then performed with antibody specific for either p65 or PKAc, followed by incubation with horseradish peroxidase-conjugated secondary antibodies. Immunoreactivity was detected by enhanced chemiluminescence. IB, immunoblot.
FIG. 7. Effects of Cr(VI) on interactions between c-Jun and CBP. A, cells were treated and immunoprecipitates (IP) were prepared as described in the legend to Fig. 6. The resulting proteins were separated by 8% PAGE, transferred to PVDF membranes, and detected by enhanced chemiluminescence. B, in a separate experiment, cells were either left untreated (control) or treated with Cr(VI) for 2 or 4 h. Total cell protein lysates were then separated by PAGE, transferred to PVDF membranes, and immunoblotted with polyclonal antibody to c-Jun. After detecting c-Jun, the blot was reprobed with monoclonal antibody to β-actin. Antibody-antigen complexes were detected by binding to secondary antibodies conjugated to horseradish peroxidase, followed by enhanced chemiluminescence.

CBP interactions. The data in Fig. 7B indicate that chromium treatment either caused no change or slightly decreased total c-Jun protein relative to β-actin. This decrease was evident only after the 4-h exposure and may have resulted from reversible, Cr(VI)-induced inhibition of protein synthesis, which has been described in this cell model (3).

DISCUSSION

Metal-induced cellular and molecular events are complex and highly concentration-dependent. Previous studies have demonstrated that metals such as Cr(VI) affect gene expression, both positively and negatively, through mechanisms that depend on induced changes in both DNA and protein structures (2, 14, 17). High concentrations of several metals, including Cr(VI), disrupt in vitro DNA binding of NF-κB primarily through modification of protein thiols, rather than changes in the DNA cis-elements (14). The data in this study suggest that, in addition to promoting structural changes in DNA or protein motifs that bind DNA, Cr(VI) alters specific protein/protein interactions that regulate transcriptional competence. In this manner, Cr(VI) may change the dynamic balance of transcription factor interactions with cofactors that are required for integrating gene expression.

NF-κB is required for transcriptional activation of a variety of inflammatory genes, and activation of NF-κB is a primary mechanism for cytokine-induced cytokine expression (18). A549 cells respond well to TNF-α stimulation with increased NF-κB activity and induction of a variety of genes, including IL-8 and uPA (Figs. 1–4). IL-8 was chosen as a model gene to study the effects of Cr(VI) on NF-κB-dependent gene expression since mutation analysis has demonstrated that removal of the NF-κB site between −94 and −71 base pairs in the promoter eliminates TNF-α and IL-1 induction of IL-8 mRNA (18, 19). IL-8 mRNA is not expressed in nonstimulated A549 cells and is not induced by treatment with Cr(VI) (15). This is consistent with the lack of a chromium effect on IL-8 expression and protein levels in other lung epithelial cell lines (20). However, pretreatment of cells with Cr(VI) prior to adding TNF-α decreased stimulated IL-8 expression. Adding Cr(VI) simultaneously with TNF-α had no effect on cytokine-stimulated IL-8 expression. This implies that either Cr(VI) or a reactive metabolite must modify its cellular target prior to activation of the cytokine signaling cascade, otherwise TNF-α signaling bypasses the inhibition. It is possible that TNF-α-stimulated association of transcriptional protein complexes protects reactive sites from modification by reactive chromium species. However, the data in Fig. 3 argue against this since cotreatment was partially effective in preventing induced expression of p55luc. This enhanced sensitivity of p55luc to chromium relative to IL-8 may reflect a greater accessibility of plasmid DNA and transcriptional complexes to reactive chromium. Nevertheless, these data indicate the selectivity of the chromium effect for NF-κB-driven expression and suggest that further investigation is needed to fully elucidate the mechanism of chromium action on more complex endogenous promoters.

The effect of Cr(VI) pretreatment on NF-κB-enhanced gene expression is limited to the level of NF-κB transcriptional competence. Cr(VI) has no effect on TNF-α signaling or its ability to activate gene transcription. Cr(VI) did not affect basal or TNF-stimulated IκB degradation (Fig. 6). Degradation of IκB is required for both release of NF-κB from cytoplasmic stores and phosphorylation of the p65 subunit by PKAc (4). Neither PKAc activity (Fig. 5) nor NF-κB translocation and DNA binding (Fig. 4) was affected by Cr(VI). These data are consistent with previous studies that demonstrated that concentrations of Cr(VI) less than 5 μM increased NF-κB nuclear translocation in Jurkat cells, whereas concentrations of 5 μM or more had no stimulatory effect (21, 22). In addition, TNF-α-induced uPA expression (Fig. 2) and expression of the generic pGL3 control plasmid (Fig. 3) were not inhibited by pretreatment with Cr(VI) unless a toxic concentration of the metal (20 μM) was added. Chromium does alter DNA synthesis (23, 24) and inhibits enzyme activities (25–27) at the higher concentrations. However, the data in this study indicated that lower concentrations of chromium did not affect cytokine signaling cascades and did not cause global, nonspecific changes in transcriptional competence, in general, or in cytokine-induced gene expression. However, these lower concentrations effectively inhibited NF-κB-dependent gene expression.

Cells transfected with the p55luc plasmid containing the NF-κB sites had increased luciferase levels above cells transfected with the empty p55luc plasmid (data not shown). Cr(VI) inhibited both basal and stimulated promoter activities in a time- and concentration-dependent manner (Fig. 3). As discussed above, this implies that reactive chromium modifies the interactions of p65/p50 with other transcriptional proteins. Acetylation of N-terminal histone tails associated with genomic DNA is crucial for transcription factor accessibility to nucleosomal templates. Transcriptional competence is conferred when p65 interacts with coactivators that bear acetyltransferase activity. CBP promotes this interaction by linking transcription factor IIB, TATA-binding protein, and histone acetyltransferases with p65 (28). It is possible that CBP is modified by a product of intracellular chromium metabolism such as Cr(III), which has been shown to bind tridentate amino acid residues and proteins (29–31). Thus, chromium could have decreased the interaction of p65 with CBP by directly modifying CBP and altering its structure or p65 recognition sequences.

The data in Fig. 7 may argue against CBP being the molec-
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In conclusion, this study has identified a novel mechanism through which Cr(VI) alters inducible gene expression. Cr(VI) inhibition of p65/CBP interactions shifts the pattern of genes expressed in response to TNF-α away from those enhanced by NF-κB. At the cellular level, loss of NF-κB transcriptional competence could have profound consequences on cell survival and expression of genes essential for innate immune responses such as essential cytokine expression. This loss of NF-κB-dependent gene expression may underlie some of the profibrotic nature of Cr(VI) in the lung.

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Phosphorylation of Ser63 and Ser73 is not. These authors speculated that phosphorylation is required to remove a repressor protein from c-Jun, which sterically hinders CBP binding (35). Additional studies are needed to determine whether this protein is labile and whether its levels are affected by the inhibitory effect of Cr(VI) on protein synthesis in this model (3). Furthermore, more detailed studies will be required to investigate the time course and stoichiometry of Cr(VI)-induced interactions of c-Jun with CBP, the mechanisms for these interactions, and whether a stoichiometric change in this interaction explains competitive inhibition of p65 binding with CBP.