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The Glucocorticoid Receptor and c-*jun* Promoters Contain AP-1 Sites That Bind Different AP-1 Transcription Factors

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The glucocorticoid receptor (GR) promoter contains several potential transcription factor recognition sites, including a putative AP-1 site. The GR promoter AP-1 site differs from the consensus AP-1 site by a nucleotide substitution, while the c-*jun* promoter contains a functionally characterized AP-1 site that varies from the consensus AP-1 site by a nucleotide insertion. Electrophoretic mobility shift assays were performed using nuclear extracts from a mouse pituitary tumor cell line (AtT-20) to test the binding capability of the AP-1 proteins, Jun and Fos, to the putative glucocorticoid receptor and the c-*jun* AP-1 sites. In addition, a comparison of the complexes formed at the GR AP-1 and c-*jun* AP-1 sites was done using antibodies specific for the Jun and Fos family members. The complexes formed with the GR AP-1 and c-*jun* AP-1 sites revealed striking differences. The GR AP-1 site formed complexes with both Jun and Fos family members. JunD was the most abundant Jun family member present, followed by JunB. cJun was absent from the complex. The amount of Fra-2 was greater than FosB in GR promoter AP-1 site complexes while Fra-1 was absent. A small amount of cFos may bind to the GR AP-1 site. In contrast to the GR promoter AP-1 site, only Jun family members were involved with complex formation on the c-*jun* promoter using AtT-20-cell nuclear extract, with JunD binding exceeding that of cJun. These results confirm previous studies suggesting that the c-*jun* promoter is stimulated solely by Jun family members. They also show preferential binding of Jun family members to different AP-1 sites present in different promoters. Finally, this study supports the hypothesis that the coordinate regulation of GR and c-*jun* gene regulation is mediated by crosstalk involving a Jun protein.

Key Words: AP-1; *fos*; glucocorticoid receptor; *jun*; promoter.

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Introduction

Steroid hormone receptors belong to a family of ligand-dependent transcription factors, and the human glucocorticoid receptor (GR) is a member of this family (Leng et al., 1996). The human glucocorticoid receptor promoter contains many putative *cis*-acting elements that were suggested using a 75% or better identity to consensus sequences (Zong et al., 1990). Among these sites is a proposed AP-1 binding site (–899 to –893). More recently, the human GR promoter (–700 to +38-bp) was found to contain 11 protein binding sites using in vitro DNA footprinting (Nobukuni et al., 1995). The AP-2 transcription factor bound to the –374 to –347 region, and five Sp1-binding sites were also identified in the proximal promoter. The other footprinted sites did not contain consensus sequences for any known transcription factors.

Fos and Jun proteins have been characterized as transcription factors that dimerize to bind DNA at the AP-1 consensus site (Angel and Karin, 1991). Fos-related proteins (cFos, FosB, Fra-1, Fra-2) must dimerize with a Jun family member (cJun, JunD, JunB) in order to bind to an AP-1 site. Unlike Fos proteins, Jun proteins can homodimerize to bind AP-1 sites. Deletions performed on the GR promoter fused to the CAT reporter gene (Govindan et al., 1991) indicated that an important site for hormone-mediated down-regulation of GR gene expression existed in the GR promoter at position –1030 to –470. The putative AP-1 site is contained in this region. However, the GR AP-1 site (TGACACA) differs from the consensus AP-1 site ($TGA^{C}/_{G}TCA$) by one nucleotide (underlined). Interestingly, the c-*jun* promoter contains an AP-1 site (TGACATCA), which differs from the consensus site by a nucleotide insertion (underlined). Unlike the GR AP-1 site, the c-*jun* AP-1 site has been functionally characterized (Angel et al., 1988).

A coordinate regulation of the glucocorticoid receptor and the c-*jun* mRNA levels has been observed in mouse AtT-20 pituitary tumor cells treated with triamcinolone acetonide (TA) (Vig et al., 1994). A comparison of the c-*jun* and GR promoters reveals that, other than the GC boxes, an AP-1 site is the only common *cis*-acting element in the two promoters. It was postulated that the AP-1 sites

in the promoters of both the GR and c-*jun* genes may be responsible for this coordinate regulation through a transcriptional interference mechanism (Jonat et al., 1990; Schüle et al., 1990; Yang-Yen et al., 1990; Vig et al., 1994).

To determine if the AP-1 site in the GR promoter might be functional, DNA electrophoretic-mobility shift assays (EMSAs) were performed to detect binding by AP-1 complexes to the proposed GR AP-1 site. Binding to the GR AP-1 site was indeed observed. Specific antibodies to the Fos and Jun protein family members were used to characterize the protein complexes bound by the different AP-1 sites. Both the consensus (collagenase) AP-1 site and the GR AP-1 site showed the involvement of Jun and Fos family members, while the complexes formed at the c-*jun* AP-1 site contained only Jun family members. This suggests that a Jun protein member is the mechanistic link in the coordinate regulation of GR and c-*jun* gene expression.

Results

Protein Titration

EMSAs were performed to compare binding of the AP-1 transcription factor to the consensus AP-1 site (TGA$^{C}/_{G}$TCA) from the collagenase gene promoter (collagenase AP-1), the putative human glucocorticoid receptor AP-1 site (XGR AP-1/YGR AP-1), the c-*jun* AP-1 site, and a mutant AP-1 site. The native GR AP-1 site was shown in preliminary experiments to form complexes with AP-1 proteins (data not shown). However, intrastrand base-pairing occurred in an unpredictable fashion in various experiments. Thus, the sequence surrounding the GR AP-1 site was changed in order to eliminate this intrastrand base pairing (Fig. 1). In addition, if both the XGR AP-1 and YGR AP-1 oligonucleotides were bound by AP-1 to about the same extent, this would indicate that the central core AP-1 sequence, rather than the flanking sequences, is important for this interaction. Nuclear extracts from AtT-20 cells shifted each of the 18-bp oligonucleotides, except for the mutant AP-1 site, in a concentration dependent fashion (Fig. 2). The collagenase AP-1 and XGR AP-1/YGR AP-1 sites showed a single specific protein-DNA complex while the c-*jun* AP-1 site showed two specific complexes with mobilities different from those obtained with collagenase AP-1 and XGR AP-1/YGR AP-1 sites. The collagenase AP-1 site appears to be the best AP-1 site. The c-*jun* AP-1 and XGR AP-1/YGR AP-1 sites were less efficient in forming complexes than the collagenase AP-1 site and the mutant AP-1 site showed only a weak complex at 20 μg of AtT-20 nuclear protein. The collagenase AP-1 (Angel et al., 1987; Lee et al., 1987) and c-*jun* AP-1 (Angel et al., 1988) sites have been shown to be functional AP-1 sites through reporter gene studies. Therefore, it appears that GR AP-1 is also a functional AP-1 site with respect to binding the AP-1 transcription factor.

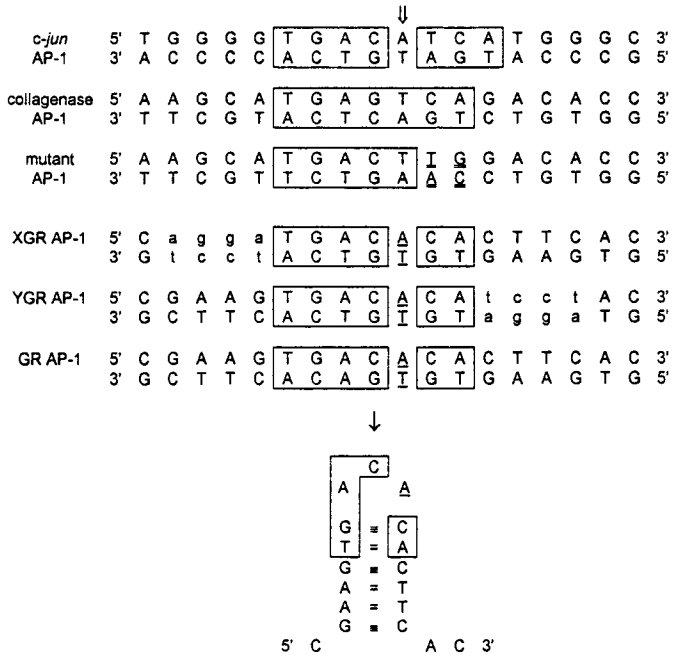

Fig. 1. Oligonucleotides used for EMSAs. Boxed nucleotides are sequences found in the consensus AP-1 site. The nucleotide insertion in the c-*jun* AP-1 site is indicated by a vertical open arrow at the top. The two nucleotides that are changed in the mutant AP-1 site are double-underlined. The nucleotide in the GR oligonucleotides that varies from the consensus AP-1 site is underlined. Lowercase letters represent nucleotides that were altered in the XGR AP-1 and YGR AP-1 from those found in the GR promoter AP-1 site. The suspected intrastrand base-pairing of the native GR AP-1 site that required modifications to generate the XGR AP-1 and YGR AP-1 oligonucleotides is shown at the bottom (only the top strand is shown).

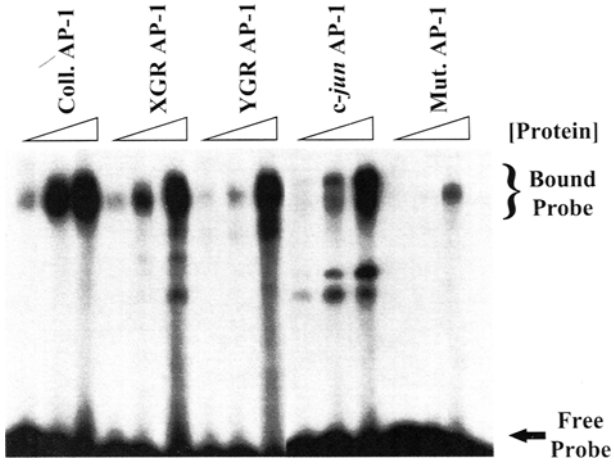

Fig. 2. Electrophoretic mobility shift assays (EMSAs) were performed using 5, 10, or 20 μg of AtT-20 nuclear protein mixed with [^{32}P]labeled oligonucleotides for the collagenase promoter AP-1 site (Coll. AP-1), the human glucocorticoid receptor promoter AP-1 site (XGR AP-1, YGR AP-1), the c-*jun* promoter AP-1 site (c-*jun* AP-1), or a mutant AP-1 site (Mut. AP-1). A probe alone lane was run as a control.

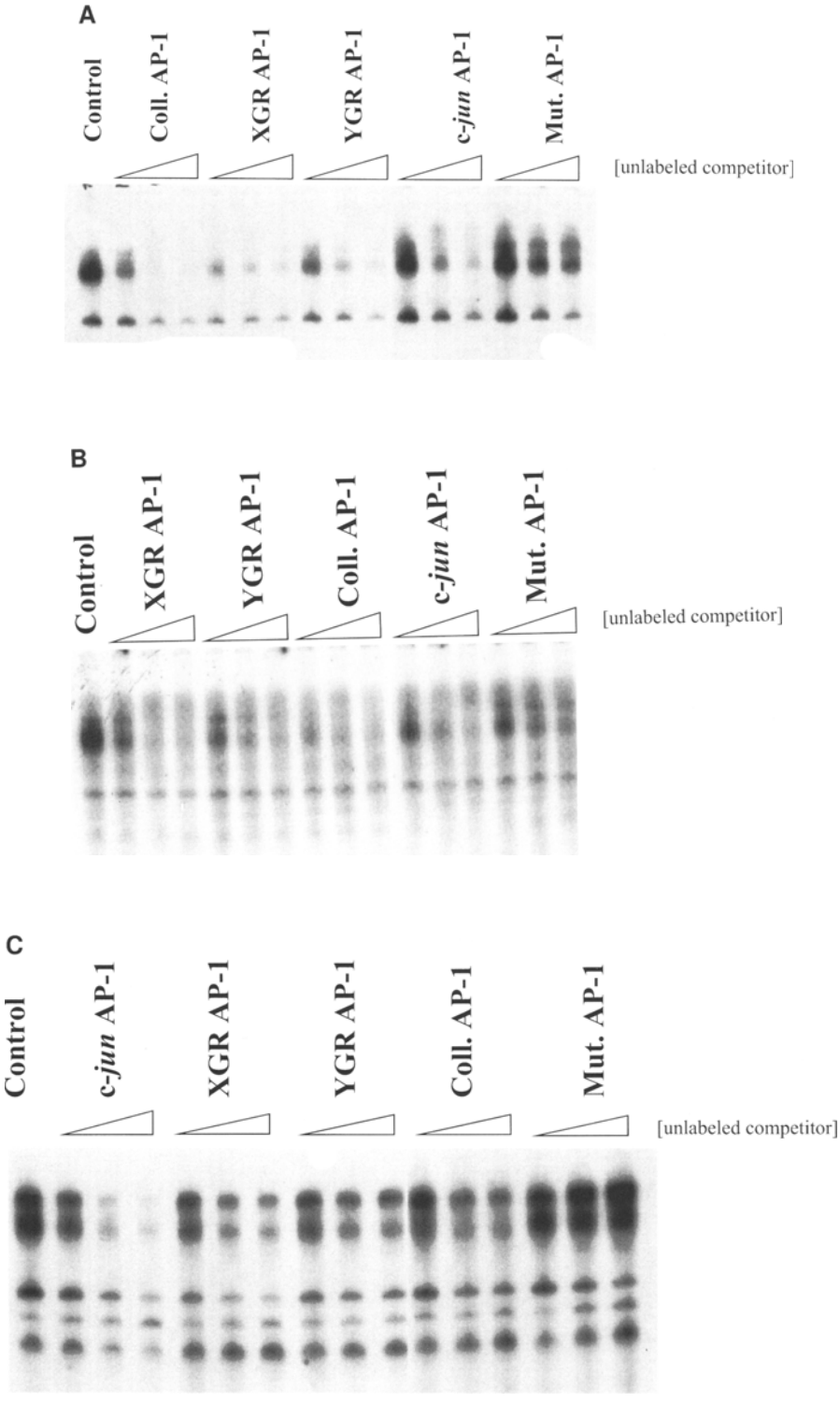

Fig. 3. EMSAs were performed with the addition of 100, 500, or 1000 times the molar excess of cold oligonucleotide containing the collagenase (consensus) AP-1, XGR AP-1, YGR AP-1, c-*jun* AP-1, or mutant AP-1 site with each of the following labeled oligonucleotides: **(A)** collagenase (consensus) AP-1 site, **(B)** XGR AP-1 site, **(C)** c-*jun* AP-1.

Competition Assays

The labeled oligonucleotides containing the collagenase AP-1, XGR AP-1, YGR AP-1, and c-*jun* AP-1 sites were competed with 100X, 500X, and 1000X unlabeled oligonucleotide to determine the relative affinity of the AP-1 sites for the AP-1 transcription complexes. The unlabeled collagenase AP-1 oligonucleotide competed most effectively for the collagenase AP-1 site, followed by the XGR AP-1 site, the YGR AP-1 site, and the c-*jun* AP-1 oligonucleotide (Fig. 3A). The unlabeled mutant AP-1 oligonucleotide competed to only a very small extent when compared to the other AP-1 sites. It appears that the XGR

AP-1/YGR AP-1 sites were about one-half as effective as the collagenase AP-1 site in displacing AP-1 binding. For the XGR AP-1 site, the order of effectiveness for competition with unlabeled oligonucleotide was: collagenase AP-1 > XGR AP-1 > YGR AP-1 > c-*jun* AP-1 >> mutant AP-1 (Fig. 3B). These data suggest that the GR AP-1 site is likely to be functional in the intact cell. Finally, the cold c-*jun* oligonucleotide was most efficient in competing binding from the c-*jun* AP-1 site (Fig. 3C), indicating specificity for this sequence compared to the XGR AP-1, YGR AP-1, and collagenase AP-1 site, which were less effective in competing for binding to the c-*jun* AP-1 site. The mutant AP-1 site (even at a 1000X excess) was not effective at all in competing for nuclear protein binding to the c-*jun* AP-1 site.

Supershift Complexes

Specific antibodies to the Jun (cJun, JunB, JunD) and Fos (cFos, FosB, Fra-1, Fra-2) proteins were used to determine the composition of the AP-1 complexes that bound to the various AP-1 sites. A pan Jun antibody and a pan Fos antibody (which were generated to highly conserved regions in Jun and Fos proteins, respectively) were also used, as were mixtures of the specific antibodies (data not shown). These experiments allowed us to dissect the components that bound to the AP-1 sites (Fig. 4).

The collagenase AP-1 and XGR AP-1/YGR AP-1 sites gave very similar results for the Jun family members (for the GR promoter, only the XGR AP-1 site results are shown here). The pan Jun antibody shifted almost all of the complexes bound to the AP-1 site, indicating that a Jun family member is a constant AP-1 component. Most of this was because of JunD, a smaller amount of activity was from JunB, while no cJun binding was observed. These results were somewhat surprising, as direct Western blotting of AtT-20 cell extracts demonstrated that cJun is an abundant Jun family member in AtT-20 cells, while JunB is present at much lower levels (Fig. 5). The pan Fos antibody shifted about 75% of the collagenase AP-1 and 100% of the XGR AP-1 complexes (Fig. 4). For the collagenase AP-1 site, the supershifting with the pan Fos antibody was mostly a result of cFos and Fra-2 binding, with nearly equivalent amounts of each activity. A mixture of the cFos and Fra-2 antibodies (data not shown) gave a pattern indistinguishable from that of pan Fos, indicating that these are the only Fos family members in AtT-20-cell nuclear extracts that bind to the collagenase AP-1 site (Fig. 4). The XGR AP-1 site bound mostly Fra-2 and FosB, with a small amount of cFos binding, as determined by supershift experiments. Thus, although subtle differences in the Fos family members that bind to the collagenase AP-1 vs XGR AP-1/YGR AP-1 sites were evident, it was clear that most of the complexes bound to these sites were heterodimers of Jun and Fos family members.

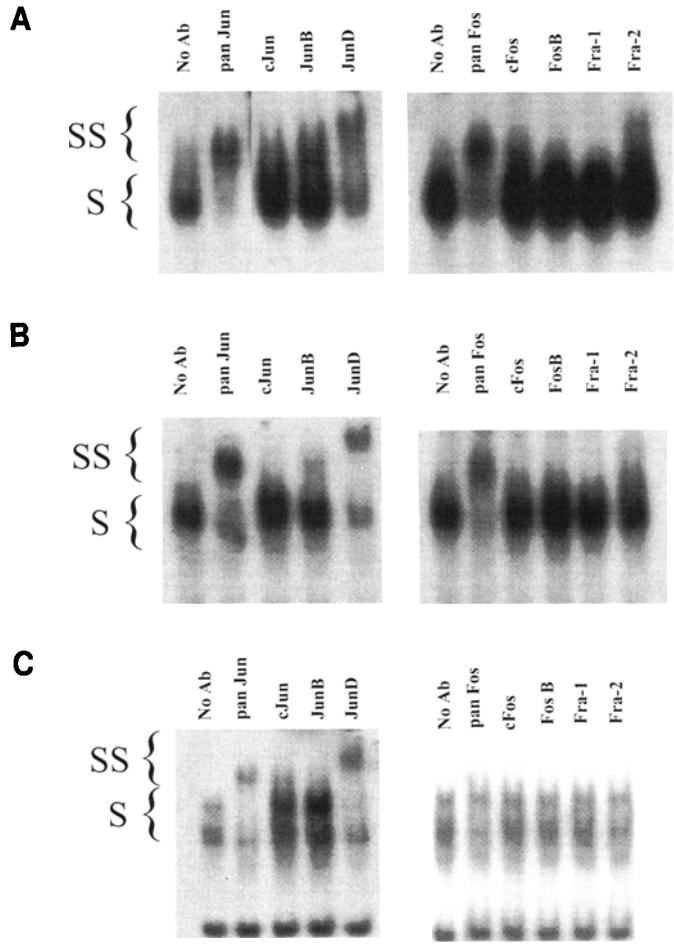

Fig. 4. Supershift assays were performed using a [^{32}P]labeled oligonucleotide containing the **(A)** collagenase (consensus) AP-1, **(B)** XGR AP-1, and **(C)** c-*jun* AP-1 sites. The complexes were supershifted with 2 μg of specific antibodies to cJun, JunB, JunD, pan Jun (which recognizes all the Jun family members), cFos, FosB, Fra-1, Fra-2, and pan Fos (which recognizes all the Fos family members). The positions of shifted (S) and supershifted (SS) complexes are indicated.

About 50% of the complexes bound to the c-*jun* AP-1 site were shifted with the pan Jun antibody and, as was true with the collagenase AP-1 site, this was mostly because of JunD binding. In some blots, a small amount of JunB binding was observed. However, in contrast to the collagenase AP-1 site, the c-*jun* AP-1 site bound no Fos family members when AtT-20 nuclear extract was used, as demonstrated using either antibodies specific for the Fos family members or the pan Fos antibody. These data clearly show a difference between the collagenase AP-1/GR AP-1 sites compared to the c-*jun* AP-1 sites. However, the pan Fos antibody does shift about 50% of the complexes bound to the c-*jun* AP-1 site when NIH 3T3 nuclear extracts are used, indicating that Fos-containing AP-1 complexes can bind to the c-*jun* AP-1 site under certain conditions and in certain cell types (Breslin and Vedeckis, data not shown).

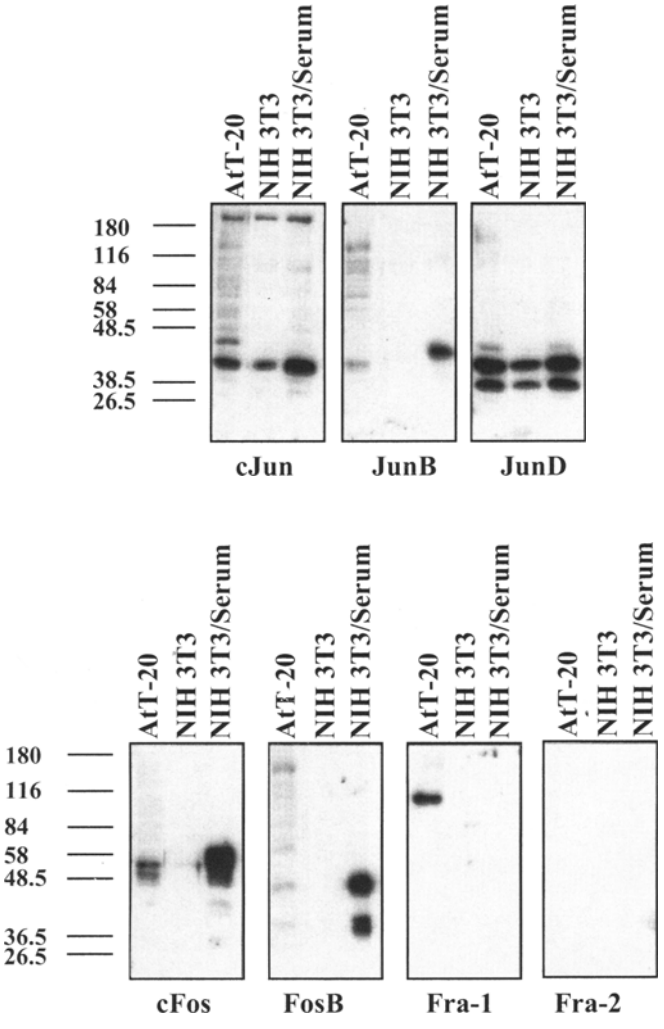

Fig. 5. Western blot analysis of AtT-20-cell nuclear extracts. AtT-20-cell nuclear extracts were subjected to SDS-PAGE and Western blotted using antibodies specific to the known Jun and Fos family members. As a positive control, nuclear extracts from serum-stimulated NIH 3T3 cells were also analyzed with each antibody.

Discussion

Our recent studies indicated a coordinate regulation of GR and c-*jun* gene expression (Vig et al., 1994). This led to a model for coupled regulation based upon transcriptional interference, and we speculated that the GR presumably inhibited binding of a Jun protein to the AP-1 sites in both the GR and c-*jun* genes (Vig et al., 1994). As the putative GR promoter AP-1 site differed from the consensus by one nucleotide, we needed to determine if it was functional in binding the AP-1 transcription factor. The present studies show that AP-1 complexes bind to the GR promoter AP-1 site. This makes it highly likely that the GR promoter AP-1 site functions as a *cis*-acting gene regulatory sequence to drive basal GR gene expression. Indeed, the identical, nonconsensus AP-1 site was discovered in the pUC19 plasmid backbone, and it was shown to be active in stimulating transcription when linked to a CAT reporter gene (Lopez et al., 1993). The fact that both the left flanking sequence (XGR AP-1) and the right flanking sequence (YGR AP-1) can be altered without destroying AP-1 binding indicates that the central core (TGACACA) sequence is the AP-1 binding site.

The use of Fos- and Jun-specific antibodies for supershift analyses clearly showed that known AP-1 transcription factors were interacting with the collagenase AP-1 and GR-promoter AP-1 sites. Virtually all of the bound complexes contain a Jun family member, either JunD or JunB. This was surprising, as Western blotting experiments showed high levels of JunD and cJun in AtT-20 cells, but very low levels of JunB. This suggests that the presence of immunogenic Jun proteins does not necessarily indicate functional (i.e., AP-1 site-binding) activity. Perhaps the phosphorylation state (Boyle et al., 1991; Lin et al., 1992) or some other modification renders these proteins active or inactive. Thus, attempting to draw conclusions regarding the physiological importance of these transcription factors based on protein levels alone could be misleading. The same situation apparently occurs with the Fos family members. While cFos is quite abundant in AtT-20 cells as determined by Western blotting (Fig. 5) and binds to the collagenase AP-1 site, it does not appear to participate significantly in GR AP-1 site binding. FosB and Fra-2 activity can account for virtually all of the Fos protein binding to the GR AP-1 site in AtT-20-cell nuclear extract. In addition, complexes of Jun members with themselves or other transcription factors appear to account for about 25% of the AP-1 binding activity in AtT-20 cells, as the pan Jun antibody supershifted all of the complexes while the pan Fos antibody only supershifted about 75%. Finally, when the JunD antibody alone was used to supershift the complex, a band of significantly slower mobility was observed. Since all Jun family members are about the same size, this could indicate that JunD is heterodimerizing with some non-Jun, non-Fos protein. Further studies are needed to demonstrate this convincingly.

Earlier studies had shown that cotransfection of a CAT reporter plasmid driven by the c-*jun* promoter AP-1 site and a high expression Jun plasmid resulted in transcriptional activation (Angel et al., 1988). These experiments were performed in mouse F9 teratocarcinoma cells, which lack endogenous cJun. It was concluded that dimers between Jun family members alone were sufficient to stimulate transcription from the c-*jun* promoter AP-1 site. The present studies directly prove that Jun proteins, and not Fos proteins, can interact with the c-*jun* AP-1 site using nuclear extracts from AtT-20 cells. Because the pan Jun antibody can only supershift about one-half the complexes on the c-*jun* AP-1 site, it is possible that some other nuclear protein(s) may be binding to this element. One possibility is that cyclic AMP response element binding-protein fam-

ily members (CREB/ATF) are binding to the c-*jun* AP-1 site. Interaction of these proteins with AP-1 sites has been shown previously (Masquilier and Sassone-Corsi, 1992; Barrett and Vedeckis, unpublished). Indeed, the c-*jun* AP-1 site closely resembles a cyclic AMP response element (CRE) in its structure. It has also been suggested that Jun/ATF-2 is the functional dimer that binds to the c-*jun* AP-1 site (Karin, 1995). However, other nuclear proteins, for example, Maf and Nrl (Kataoka et al., 1994; Kerppola and Curran, 1994), can also interact with Fos and Jun and bind to an AP-1 site or a CRE, so that additional experiments are needed to resolve this question.

Clearly, the relative interactions of these nuclear proteins with different AP-1 sites and the varied composition of the complexes and their activity (stimulatory or inhibitory) can result in a highly complex, versatile, and precise control of gene expression. For example, the cFos/cJun dimer is very potent in stimulating gene transcription, while, in certain cell types, complexes containing JunB or other Fos family members actually can suppress transcription from AP-1 sites (Angel et al., 1991; Nakabeppu and Nathans, 1991; Yen et al., 1991; Suzuki et al., 1992; Schlingensiepen et al., 1993). Similarly, CREB binding to AP-1 sites also suppresses transcriptional activation by Jun (Masquilier and Sassone-Corsi, 1992). The composition and activation of the various AP-1 transcription factors in different cell types could lead to cell-type specific differences in the regulation of the same gene. A recent study of glucocorticoid-, cJun-, and Fos family member-mediated regulation of the neurotensin/neuromedin N gene is an example of the complex interactions of different cellular transcription factors on gene expression (Harrison et al., 1995).

The present study adds further credence to the model for coordinate GR and c-*jun* gene regulation in corticosteroid-treated AtT-20 cells (Vig et al., 1994). The initial model depicted cFos/cJun dimers binding to both the GR promoter and c-*jun* promoter AP-1 sites. In view of the present results, this model requires revision. First, although the GR promoter AP-1 sites are bound by heterodimers containing a Jun and a Fos family member, cFos and cJun do not appear to be involved. Antibodies specific to these two proteins were ineffective in supershifting any of the complexes, while the pan versions were active. The Jun family member is either JunD or JunB, while the Fos family member is FosB or Fra-2. The precise composition of these complexes (FosB/JunD; FosB/JunB; Fra-2/JunD; Fra-2/JunB) has not yet been determined.

The c-*jun* promoter AP-1 site is not bound by any Fos family member-containing complexes by AtT-20-cell nuclear extracts. About one-half contain Jun family members (JunD/JunD, JunD/JunB, and/or JunB/JunB, or, JunD or JunB heterodimerized to some other non-AP-1 protein). The other one-half of the complexes bound to the c-*jun* AP-1 site that are not supershifted by the pan Jun antibodies may contain only non-AP-1 proteins. Because of the similarity between the c-*jun* promoter AP-1 site (TGACATCA) and the consensus binding site for members of the CREB/ATF family (TGACCTCA), the latter are the most likely candidates for the binding of non-AP-1 proteins to the c-*jun* promoter AP-1 site. Preliminary results support this interpretation (Barrett and Vedeckis, unpublished).

These results do not substantially alter the model for coordinate regulation of GR and c-*jun* gene expression after steroid hormone treatment. We still propose that the transformed GR concomitantly suppresses GR and c-*jun* gene expression via transcriptional interference. However, rather than the GR forming an inactive complex with cJun, it now seems more likely that JunD or JunB may be inactivated by heterodimerization with the GR. This would reduce the number of active AP-1 transcription factors that drive both GR and c-*jun* gene transcription. We previously presented arguments for why a Fos family member was not the AP-1 target for transcriptional interference (Vig et al., 1994). The present results indicating that there are no Fos family members involved in binding to the c-*jun* promoter AP-1 site in AtT-20 cells further strengthens this conclusion, as Jun family members are the only common proteins that bind to both the GR promoter and c-*jun* promoter AP-1 sites.

Materials and Methods

Cell Culture

Mouse AtT-20 pituitary tumor cells (Suzuki et al., 1992; Harrison and Yeakley, 1979) were grown in Dulbecco's modified Eagles medium/F12 (DMEM/F12) (GIBCO) supplemented with 10% newborn calf serum (Bio Whittaker). Spinner cultures were grown to 0.5×10^6 cells/mL in a 1-L culture.

Preparation of Nuclear Extract

Nuclear extracts were prepared as described elsewhere (Digman et al., 1983). Briefly, the AtT-20 cells were harvested by centrifugation and washed once with phosphate-buffered saline and once in hypotonic buffer. The cell pellet was homogenized in hypotonic buffer (10 m*M* HEPES, pH 7.9, 1.5 m*M* $MgCl_2$, 10 m*M* KCl, 0.2 m*M* phenylmethylsulfonylfluoride [PMSF], 0.5 m*M* dithiothreitol [DTT]) in a Dounce homogenizer with a "B" pestle. This was centrifuged (3300*g*, 15 min), and the nuclear fraction was extracted with 20 m*M* HEPES, pH 7.9, 25% glycerol, 1.5 m*M* $MgCl_2$, 300 m*M* KCl, 0.2 m*M* EDTA, and 0.5 m*M* DTT. The nuclear extract was dialyzed against 100 vol of dialysis buffer (20 m*M* HEPES, pH 7.9, 20% glycerol, 100 m*M* KCl, 0.2 m*M* EDTA, 0.2 m*M* PMSF, and 0.5 m*M* DTT) for 1 h at 4°C. The nuclear extract was flash frozen in 50-μL aliquots in liquid nitrogen and stored at −70°C. Protein determinations were performed using a bovine serum albumin (BSA) standard and the Bio-Rad (Hercules, CA) DC Protein Assay kit.

Electrophoretic Mobility Shift Assays

Electrophoretic mobility shift assays (Fried and Crothers, 1981; Garner and Revzin, 1981) were typically done with 10–15 μg of AtT-20 nuclear protein in a 15-μL reaction volume. All oligonucleotides were synthesized by the LSU Medical Center Core Laboratories. Binding conditions were: 13 m*M* HEPES, pH 7.9, 67 m*M* KCl, 12% glycerol, 0.1 m*M* PMSF, 0.1 m*M* EDTA, 0.3 m*M* DTT, 2 μg poly dIdC·poly dIdC (Pharmacia, Uppsala, Sweden), and 40,000 cpm of a γ[^{32}P]ATP (Amersham [Arlington Heights, IL], 3000 Ci/mmol) end-labeled, double-stranded oligonucleotide. Binding was done for 15 min at room temperature. Protein-DNA complexes were separated on 4% polyacrylamide gels in a 22.5 m*M* Tris-borate (pH 8.0) and 0.5 m*M* EDTA buffer. The gels were run at room temperature at 35 mA/gel. For supershift assays, after the 15 min room temperature incubation, 2 μL of rabbit polyclonal antibodies (Santa Cruz Biotechnologies [Santa Cruz, CA], 1 mg/mL) for cJun (#sc-45X), pan (c)Jun (#sc-44X), JunD (#sc-74X), JunB (#sc-46X), cFos (#sc-52X, pan (c)Fos (#sc-253X), FosB (#sc-48X), Fra-1 (#sc-183X, or Fra-2 (#sc-171X) were added. The samples were incubated on ice or at room temperature for 1 h and separated on a 4% polyacrylamide gel at 4°C on the Bio-Rad Miniprotean II system. Gels were dried and exposed overnight to Hyperfilm MP (Amersham) with an intensifying screen. The probes containing the AP-1 sites are diagrammed in Fig. 1. Complementary oligonucleotides were mixed in equimolar concentrations and incubated at room temperature to anneal. Competition assays were performed by adding unlabeled oligonucleotide at 100-, 500-, and 1000-times the molar excess of the labeled oligonucleotide prior to the addition of the nuclear extract.

Western Blot Analysis

Western blotting of nuclear extracts was performed as described previously (Angel et al., 1988). Nuclear extracts were mixed with an equal volume of 2X Laemmli sample buffer, and 10 μg of protein were submitted to SDS-PAGE. The same antibodies used for supershift analysis were utilized for Western blotting, at a concentration of ~0.5 μg/mL (depending on the specific antibody) of primary antibody The blots were processed and analyzed using enhanced chemilunninescence (ECL kit; Amersham).

Image Analysis

X-ray films with the ECL data were scanned with a Hewlett Packard Model IIcx flat bed scanner using HP DeskScan II (Microsoft Windows version) at a resolution of 400 dpi. Figures were printed using a Lexmark Optra R laser printer at 1200 dpi resolution.

Note Added in Proof

A recent paper has appeared (Kamei, et al., *Cell* **85,** 403–414, 1996) that indicates a crucial role for CREB-binding protein (CBP) in GR-mediated upregulation of gene expression and in downregulation of AP-1 activity. This does not change the fundamental observations or conclusions of the present paper. However, rather than transcriptional interference being due to GR/Jun heterodimers, the formation of GR/CBP heterodimers may deplete the level of CBP below that necessary for coactivating AP-1 transcription factor complexes.

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