Expression of Human Glucocorticoid Receptor Gene and Interaction of Nuclear Proteins with the Transcriptional Control Element*

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We have identified sequences responsible for the expression of the human glucocorticoid receptor gene (GR gene) using a set of 5' promoter deletion mutants in HeLa, human placenta, and human breast tumor (MCF-7) cells. The chimeric gene construct −892 5'-GAAGTGACACACTTC−3' −878-CAT was sufficient for high level of expression in HeLa and placenta cells in culture. Deletion of palindromic sequences decreased levels of GR expression in these cells. By oligonucleotide-affinity chromatography with the palindromic glucocorticoid receptor enhancing factor-binding element (GREFE), we have isolated from human placenta nuclear extract two novel proteins glucocorticoid receptor enhancing factors 1 and 2 (GREF1 and GREF2), with apparent molecular masses of 80 and 62 kDa, respectively. These proteins, similar to the DNA-binding autotranslational Ku are, like Ku, heterodimers of polypeptide subunits p80 and p62, immunologically related to factors binding to proximal sequence element 1 element in the promoter of small nuclear RNA (PSE1) and transferrin receptor enhancing factors. Both Ku80 and Ku70 polypeptides were present in high concentrations in human placenta and HeLa cells. In MCF-7 cells, however, only a high level of p62 was detected. While cotransfection of pcDNA-Ku80 with pHGR (−892 to −878)-CAT potentiated the expression of CAT, introduction of pcDNA-Ku70 did not affect the expression of CAT in transfected MCF-7 cells. UV cross-linking analysis showed that only GREF 1 contacted DNA directly. Super-shift assays with monoclonal antibodies Ab 111 (Ku80) or Ab N3H10 (Ku70) showed a direct interaction of GREF 1 and GREF 2 heterodimers with the palindrome. Partial peptide fingerprinting of GREF 1 and GREF 2 using a-chymotrypsin and immunoblotting with Ab 111 and Ab N3H10 confirmed their identities as Ku80 and Ku70, respectively.

The regulation of transcription in eukaryotes is dictated by the cooperative interaction between various sequence-specific DNA-binding proteins (1, 2). A number of these trans-acting factors at the start of transcription have been identified (3, 4), some of which are expressed in a number of cell types. The activation of gene transcription is an ability associated with a number of eukaryotic DNA-binding activators and variation in gene expression is achieved by modulating the activity of these DNA-binding transcription factors. The glucocorticoid receptor (GR), a member of the nuclear receptor superfamily, mediates the actions of glucocorticoids by direct interactions and regulates the transcription of glucocorticoid responsive genes (5–9). Although the GR is widely distributed in nearly all cell types and has an essential role in cell metabolism and growth, its regulatory role in a number of biological events in tissues such as liver and placenta is not yet well understood.

The human placenta is a hormone responsive tissue in which excessive levels of glucocorticoids have deleterious effects on the fetus that lead to impaired fetal growth and teratogenesis, and in the adult, may predispose the individual to hypertension (10–12). We were primarily interested in studying the transcriptional regulatory elements present in the promoter region and in examining the promoter activity (13–16). Considering that the glucocorticoid effects are mediated by GR, we have constructed a series of 5' deletion mutants containing the promoter region of the hGR gene fused to chloramphenicol acetyltransferase (CAT) chimeric gene and introduced them into human placenta cells in culture.

The human GR gene contains the palindromic sequence motif 5'-GAAGTGACACACTTC-3' (−892 to −878) in the regulatory region. We designate this sequence as GREFE, the glucocorticoid receptor enhancing factor-binding element. The human GR promoter sequence reported (14, 17, 18) corresponds to the IC promoter of the mouse GR and promoters 1A, 1B, and IC were shown to have varying activities in different cell lines and tissues (19). Deletions of various sequence of the regulatory region of the hGR gene and subsequent analyses point to a differential promoter activity in various cells (13, 15, 17–21).

The hGR promoter lacks the classical TATA box, but TATAA and CAAT boxes are present in the regulatory region located −54 nucleotides upstream of the first base in the cDNA (22). Previously, we identified by S1 nuclease mapping and primer extension the authentic GR transcription start site in the isolated genomic fragment (13, 14, 20).

To characterize the proteins binding to this palindromic recognition site, we have extracted nuclear proteins from human placenta and analyzed their interaction with the synthetic GREFE. Here, we describe an initial characterization of these proteins by DNA-protein interaction, purification by sequence-specific interaction chromatography, resolution of the UV cross-linked DNA-protein complexes by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and by Western blotting with anti-Ku antibodies (23). Furthermore, these polypep-
tides were identified as Ku80 and Ku70 by cleavage with site-specific endopeptidase, Western blotting, and immuno detection with monoclonal antibodies (24, 25). Finally, we demonstrate the sequence-specific interaction of Ku80 and Ku70 with the regulatory sequence by mobility-supershift assays using monoclonal antibodies against either Ku80 or Ku70.

**MATERIALS AND METHODS**

Cell Culture—Human Placenta (3A-SUB-E, CRL-1583), HeLa, and MCF-7 cells were grown in MEM medium (Sigma) supplemented with 10% fetal calf serum. Human prostate cancer cells LNCaP were propagated in RPMI medium without phenol red.

Buffers—Buffer A: 40 mM Tris-HCl, pH 7.5, 0.25 mM sucrose, 5 mM EDTA, 7 mM β-mercaptoethanol, 2.5 mM phenylmethylsulfonyl fluoride (PMSF). Buffer B: 25 mM HEPES, pH 7.5, 5 mM KCl, 0.5 mM MgCl₂, 1 mM DTT, 1 mM PMSF, 10% glycerol. Buffer C: Buffer B + 0.01% Nonidet P-40. Buffer D: 25 mM HEPES, pH 7.5, 40% glycerol, 0.01% Nonidet P-40, 1 mM DTT, 2 mM EDTA, 1 mM PMSF. Buffer E: 25 mM HEPES, pH 7.5, 10% sucrose, 0.01% Nonidet P-40, 1 mM DTT, 2 mM EDTA, 1 mM PMSF. Buffer F: 45 mM Tris, 45 mM boric acid, and 1.25 mM HEPES, 10% sucrose. Buffer G: 12 mM HEPES, pH 7.8, 1 mM DTT, 0.5 mM EDTA, 60 mM KCl, 1 mM PMSF, 10% glycerol. Buffer H: 50 mM Tris-HCl, pH 7.9, 6 mM MgCl₂, 1 mM DTT, 40 mM ammonium sulfate, 15% glycerol, 1 mM EDTA. Buffer I: (stock solution is 3 M NaAc, pH 5.2, with 500 mM EDTA. After dissolution of the sucrose, the pH was adjusted to 7.0 with 1 N NaOH (10.5 M) and stirred overnight at 4°C. The precipitated nuclear proteins were collected by centrifugation at 26,000 rpm in an SW-28 rotor at 4°C for 20 min. The supernatant was decanted into an Erlenmeyer flask and centrifuged at 26,000 rpm for 20 min at 4°C to collect the crude nuclear fraction. The nuclei were washed once by resuspension in 500 ml of Buffer E and centrifugation. The nuclear pellet was resuspended in a total volume of 400 ml of Buffer G containing 10 mM NaCl, 0.01% Nonidet P-40, 1 mM DTT, 2 mM EDTA, 1 mM PMSF. The solution was centrifuged at 200,000 x g, diluted in 25 ml of Buffer G, and filtered through 3 layers of cheesecloth and centrifuged to collect the nuclei. The nuclei were resuspended in 500 ml of Buffer E and centrifugation. The nuclear pellet was resuspended in a total volume of 400 ml of Buffer G containing 10 mM NaCl, 0.01% Nonidet P-40, 1 mM DTT, 2 mM EDTA, 1 mM PMSF. The solution was centrifuged at 200,000 x g, diluted in 25 ml of Buffer G, and filtered through 3 layers of cheesecloth and centrifuged to collect the nuclei.

Preparation of the Nuclear Extract from Placenta—Two placentas weighing about 600 g were homogenized with a Waring blender in 1,200 ml of Buffer A. The homogenate was centrifuged at 1,500 rpm for 15 min at 4°C to collect the crude nuclear fraction. The nuclear extract was either prepared immediately from the crude nuclear fraction or frozen at −20°C for 4–6 weeks. The crude nuclear pellet was resuspended in 500 ml of Buffer B, centrifuged to collect the nuclear fraction and resuspended again, this time in 500 ml of Buffer C, filtered through 3 layers of cheese cloth and centrifuged to collect the nuclei. The nuclei were washed once by resuspension in 500 ml of Buffer E and centrifugation. The nuclear pellet was resuspended in a total volume of 400 ml of Buffer G containing 10 mM NaCl, 0.01% Nonidet P-40, 1 mM DTT, 2 mM EDTA, 1 mM PMSF. The solution was centrifuged at 200,000 x g, diluted in 25 ml of Buffer G, and filtered through 3 layers of cheesecloth and centrifuged to collect the nuclei.

Preparation of the Affinity Matrix—The oligonucleotide 5'-GGGTGAGAATACACATTACACA-3' and the complementary 5'-TGGTGAATTCAACCTCACA-3' were synthesized by Oligo Express, BioCan Scientific at a 0.5 μmol scale and purified by reverse phase high performance liquid chromatography on a C-18 column (Phenomenex). The four guanosines at the 5' end was found to be suitable for coupling to the CNBr-activated Sepharose 4B. 1.25 mg each of the oligonucleotides were annealed together in Sequence buffer (500 μl) and an aliquot of 0.5 μg was labeled with ['32P]ATP to determine the efficiency of coupling to CNBr-activated Sepharose 4B. For coupling, 1.5 g of activated Sepharose was used and procedures were as described by the manufacturer.

Expression and Interaction with Transcriptional Control Elements—To analyze the interaction of intact hGR gene fragment containing the authentic nuclear protein interaction site, a 280-bp base pair hGR gene fragment between SmaI (−1030) and SacI (−750) was purified and labeled at the dephosphorylated 5' Smal end (13–15, 20). An aliquot of 10,000 cpm was incubated with the purified protein and incubated for 15 min. The protein-DNA complexes were resolved on 5% nondenaturing polyacrylamide gels using 22.5 mM Tris, 22.5 mM borate acid, and 0.5 mM EDTA, pH 8.0 (0.25 × TBE). The specific interaction of the proteins was assayed by deleting the proteins in the incubates with monoclonal antibodies and immunoblotting as described above.

Affinity Chromatography—The nuclear extract was thawed and diluted to 2.5 mg/ml in protein concentration with Buffer G. The solution was clarified by centrifugation at 50,000 rpm in a Beckman Ti-70 type rotor for 30 min at 4°C and loaded on the affinity matrix at 1 ml/min. The flowthrough was recirculated a second time at the same rate at the same time the elution was collected and the column was washed with 100 volumes of Buffer G, followed by 10 volumes of Buffer G containing 120 mM NaCl and the column was developed with a linear gradient (25 ml each) of 0.1–1 M NaCl in Buffer G. Fractions of 1 ml were collected and analyzed for specific DNA binding activity by gel mobility shift assay. Subsequently, the purified proteins were resolved on 10% SDS-polyacrylamide gel electrophoresis, and Western blotting with human anti-Ku autoantibodies (27, 28). The purity of the eluted proteins was determined by SDS-polyacrylamide gel electrophoresis and silver staining.

Analysis of DNA-Protein Interaction with Intact hGR Gene Segment—To analyze the interaction of intact hGR gene fragment containing the authentic nuclear protein interaction site, a 280-base pair hGR gene fragment between SmaI (−1030) and SacI (−750) was purified and labeled at the dephosphorylated 5' Smal end (13–15, 20). An aliquot of 10,000 cpm was incubated with the purified protein and incubated for 15 min. The protein-DNA complexes were resolved on 5% nondenaturing polyacrylamide gels using 22.5 mM Tris, 22.5 mM borate acid, and 0.5 mM EDTA, pH 8.0 (0.25 × TBE). The specific interaction of the proteins was assayed by deleting the proteins in the incubates with monoclonal antibodies and immunoblotting as described above.

Supershift Assays—The purified proteins from placenta were incubated either with 1 μl of anti-human Ku70 monoclonal antibodies (N3H10) or anti-human Ku80 monoclonal antibodies (111) for 15 min at room temperature prior to the addition of labeled DNA as indicated (monoclonal antibodies were generously provided by Dr. W. Reeves, University of North Carolina, Chapel Hill). To further define the specificity, the antigen-antibody complexes were immunoprecipitated on protein A-Sepharose and the resulting supernatants were used in DNA-protein interaction assays.
The expression of hGR in various target tissues and cells is subject to multiple controls. We have examined the importance of the 5'-regulatory sequences in the promoter region of the hGR gene in an effort to identify and to characterize factors involved in the regulatory pathway (Fig. 1).

Identification of DNA Sequences Directing the Tissue-specific Expression of GR Gene—To identify sequences involved in the regulation of hGR gene, MCF-7, HeLa, and human placenta cells were cotransfected with hGR deletion mutants fused to the CAT reporter gene. In extracts prepared from MCF-7 cells transfected with pBL-CAT (Fig. 2, lane 1), pHGRΔ908-CAT (lane 2), pHGRΔ830-CAT (lane 3), and pHGRΔ747-CAT (lane 4), reporter gene transcription was only basal. In placenta and HeLa cells, CAT expression also remained basal with control pBL-tkCAT (Fig. 2, lanes 5 and 9) and the deletion mutant pHGRΔ747-CAT (lanes 8 and 12). However, there was a 5-fold increase in CAT activity expressed by the chimera pHGRΔ830-CAT (lanes 7 and 11) in placenta and HeLa cells compared to the levels observed with the control pBL-CAT construct. Similarly, in placenta and HeLa cells cotransfected with pHGRΔ908-CAT (lanes 6 and 12), there was a 12-fold increase in CAT activity expressed by the chimera pHGRΔ908-CAT (lanes 6 and 12).
in CAT expression. These results indicate that sequences between −830 and −908 5′ to the GR promoter are involved in the cell specific and enhanced expression of the GR gene.

As shown in Fig. 3, alignment of sequences in the transcriptional control elements of various genes reveals the presence of a unique palindrome adjacent to a putative Sp1 binding site (13, 14, 20). To further delineate the sequences involved in the GR expression, we fused a single copy of this palindrome, GREFE, to the heterologous CAT reporter plasmid pBL-tkCAT. In human MCF-7, LNCaP, HeLa, and placenta cells transfected with control plasmid pBL-tkCAT, CAT expression was at comparable constitutive levels (Fig. 4, lanes 1, 2, 5, and 6, respectively). In MCF-7 and LNCaP cells (Fig. 4, lanes 3 and 4), CAT expression by the palindrome-containing reporter plasmid pHGR-CAT (−892/−878) did not rise above basal levels. In HeLa (lane 7) and placenta cells (lane 8), an 8-fold increase in CAT activity was observed with pHGR-CAT (−892 to −878), confirming that the sequences between −892 and −879 function as enhancer sequences of GR gene expression.

Purification of the Factor(s) by Sequence-specific Affinity Chromatography—The enriched nuclear extract from human placenta was clarified and depleted of the unspecific adsorbed proteins. Under the affinity chromatography conditions described above, 90% of the factor(s) bound to the stationary matrix. To detect and to visualize the proteins, fractions were analyzed by SDS-polyacrylamide gel electrophoresis and silver staining (Fig. 5A). Next, we performed UV cross-linking analyses followed by SDS-polyacrylamide gel electrophoresis and autoradiography (Fig. 5B) and finally examined the interaction of the factor(s) with the synthetic palindrome (Fig. 5, C and D) and conducted Western blotting (Fig. 5E).

UV Cross-linking Studies—To define the interaction of specific factor(s) with the palindrome, we incubated samples of various fractions with the labeled oligonucleotide, GREFE. The separation of the complexes on acrylamide gels (Fig. 5C) followed the elution pattern of the factors from the affinity column. The proteins were characterized using a combination of DNA-binding and UV cross-linking analyses. The resolution of the UV cross-linked complexes on a SDS-polyacrylamide gel, showed the presence of a major labeled band at 80 kDa (Fig. 5B), which comigrated with the silver-stained 80-kDa protein band (Fig. 5A, lanes fractions 3, 5, 7, 9, 11, and 13). Two additional low molecular bands with reduced intensity at 66 and 50 kDa (Fig. 5B) were also visible. The reduction in the intensity of the retarded complexes in the presence of 100-fold molal non-radioactive competitors in parallel incubations, confirmed the specificity of the interactions (Fig. 5D). The two proteins will be henceforth designated as GREF1 (80 kDa) and GREF2 (62 kDa).

Immunological Similarities of GREF1 and GREF2 to Human Ku-Autoantigen—We have characterized the proteins GREF1 and GREF2 from human placenta, with the information available from previous investigations on TREF1 and...
We resolved nuclear proteins from human placenta (Fig. 5, lane 1), HeLa cells (lane 2), oligonucleotide affinity eluted fractions 7, 9, 11, and 13 (lanes 3-5, and 6, respectively) and Ku70 expressing bacteria (lanes 1, 2, 3, and 4) with monoclonal antibodies. The Western blot analysis confirmed the specificity of the antibodies against Ku70. Nuclear extract from CV-1 cells (lane 8) and LNcAP (lane 10) did not cross-react with the human serum and MCF-7 cells contained only the Ku70-like antigen (lane 9). The antibodies in the human serum reacted with the 70-kDa polypeptide much more strongly than with the 80-kDa protein. The high molecular weight band cross-reacting with the antibodies may be the heterodimers of Ku80 and Ku70-like proteins (lanes 1–7) in placenta and HeLa cells.

Partial Peptide Mapping with Monoclonal Antibodies—To identify the isolated factors, we performed partial cleavage with a-chymotrypsin and determined the cross-reactivity of the peptide fragments by Western blotting using monoclonal anti-
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The ability of synthetic GREFE to interact with factors present in nuclear extracts of various cells was determined by gel mobility-shift assay. The nuclear extract from human placenta cells in culture showed a strong complex formation (Fig. 7, lanes 1–5). The presence of a gel-shifted band following the addition of 100-fold molar excess of non-labeled probe, demonstrated the specificity of this interaction (lane 3). Under identical experimental conditions, the gel-shifted complexes associated with nuclear extract from MCF-7 cells migrated faster (lanes 4 and 5) than the complexes observed with the placenta nuclear extract (lanes 2 and 3). Samples of HeLa cell nuclear extract formed heavier complexes (lanes 6 and 7) which migrated with identical mobility as the complexes observed with placenta extract. pET-70 produced in bacteria, formed faster migrating complexes (lanes 10 and 11) which were much less intense, indicating that these were lighter than the larger complexes with placenta and HeLa nuclear extracts. A lighter band of fast migrating complexes was observed with the nuclear extract from human prostate cancer cell LNCap (lanes 12 and 13) and no complexes were visible with either CV-1 (lanes 8 and 9) or COS-1 nuclear extracts (lanes 14 and 15). Similarly, no complexes were observed using labeled synthetic glucocorticoid responsive palindrome (GRE) in control experiment with the purified factors from placenta (not shown).

The Cross-reaction of Purified Factors with Anti-human Ku70 and Ku80 Monoclonal Antibodies—To assess the DNA-protein interaction with intact hGR gene segment containing the protein-binding site, we isolated a 280-base pair hGR gene fragment between SmaI (−1030) and SacI (−750) and used it in DNA-protein interaction assays. Specific interaction of the labeled DNA (Fig. 8A, lane 2) was abolished by immunosorbing the factors either with anti-Ku70 (Fig. 8A, lane 3) or anti-Ku80 (lane 4) and protein A-Sepharose. The specificity of the interaction was assayed by the addition of a 10-fold (lane 5) and 100-fold (lane 6) molar excess of non-labeled DNA as competitor. Addition of a nonspecific IgG in the depletion experiment did not affect the formation of specific DNA-protein complexes (Fig. 8B, lane 7).

The ability of monoclonal antibodies to interact with specific proteins can be used to characterize the DNA-protein interaction in supershift assays. As shown in Fig. 8B, compared to the complexes observed in the absence of monoclonal antibodies...
Although the addition of a nonspecific IgG did not affect the migrating complexes (Fig. 8B, lane 2) but no increase in transcription was observed by cotransfection with either Ku70 or Ku80 expression vectors alone (lanes 7 and 8, respectively). The results were considerably different in assays with the intact pHGR2.7-CAT (lanes 9–12) and pGREFE-CAT (lanes 13–16). Transcription of the CAT gene was stimulated considerably by pcDNA Ku80 (lanes 12 and 16) but not by pcDNA Ku70 (lanes 11 and 15). The control pcDNA1 cotransfected cells demonstrated the basal transcription levels (lanes 10 and 14).

**DISCUSSION**

DNA Sequences between −908 and −830 in the Regulatory Region Determine the Levels and the Tissue-specific Expression of hGR Gene—The regions of hGR gene promotor required for efficient expression are unknown. To identify sequences involved, we determined the responsiveness of the chimeric...
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Table 1. Summary of the cotransfection experiments with the chimeric CAT reporter genes in MCF-7 cells.

| Gene       | Transfection | pCH110 | pBL-CAT | pHGR2.7-CAT | pHGRΔ830-CAT | pHGRΔ830/ΔS0-CAT | pDNA1 | pDNA1-Ku80 | pDNA1-Ku70 | pHGRΔ830CAT | pDNA1 | pDNA1Ku80 | pHGRΔ830CAT | pDNA1 | pDNA1Ku80 | pHGRΔ830CAT | pDNA1 | pDNA1Ku80 |
|------------|--------------|--------|---------|-------------|--------------|------------------|-------|------------|-------------|-------------|-------|-----------|-------------|-------|-----------|-------------|-------|-----------|
| MCF-7      |              | 1      | 2       | 3           | 4            | 5                | 6     | 7          | 8           | 9           | 10    | 11        | 12           | 13    | 14        | 15           | 16    | 17        |

Fig. 10. Reconstitution of hGR gene expression in MCF-7 cells. Semiconfluent MCF-7 cells were co-transfected with pBL-CAT (lanes 1–4), MMTV-CAT (lanes 5–8), pHGR2.7-CAT (lanes 9–12), and pGREFE-CAT (lanes 13–16) with pCH110 as described. The transient transfection was performed either in the presence of 5 µg of pDNA1 (lanes 2, 5, 6, 10, and 14), 5 µg of pDNA1 Ku70 (lanes 3, 7, 11, and 15), or 5 µg of pDNA1 Ku80 (lanes 4, 8, 12, and 16). As an internal control, the ability of endogenous human progesterone receptor to induce the transcription of MMTV-CAT was determined by treating the transfected cells with 1 µM R5020 (lane 6). The acetylated chloramphenicol derivatives were visualized by autoradiography and the radioactivity was determined by scintillation counting. The values are the average of triplicate experiments and shown are the results of complementation of Ku80 into MCF-7 cells and the induction of GR gene transcription activation involving the palindrome.

HGR-CAT constructs by transient cotransfection in MCF-7, HeLa, and human placenta cells. The high levels of CAT activity observed with pHGRΔ830-CAT and the decline with pHGRΔ830-CAT in human placenta and HeLa cells, first indicated the presence of a regulatory mechanism between these deletions that involved cis-acting sequence motifs with a putative Sp1 binding site at the 3’ end (29).

Sequence Homologies between the HGR, Promoter and Promoters of Other Genes—The GR gene sequence palindromic responsible for the high levels of GR expression in human placenta and HeLa cells is distinct from sequences described previously. However, it shares common features with transcriptional control elements present in a number of other genes. The GR gene sequence palindrome which constitutes the binding site for the GR protein and the palindrome. Although, the transcription factors CREB, the Jun-Fos/Fra complex of AP-1, and MLTF/USF appear to recognize related sequence elements (24, 25, 47–56), there is a marked size difference between these factors and GREF1 (80 kDa) and GREF2 (62 kDa). In human placenta extracts, the presence of protein PSE1, a dimer of 83- and 73-kDa subunits, was demonstrated. This PSE1 had immunological and sequence relationships with p70 and p86 of the Ku complex (57).

Heterodimers of Ku80 and Ku70-like Proteins Regulate hGR Gene Transcription in MCF-7 Cells—The cotransfection of pDNA1-Ku70 with pGREFE-CAT did not elevate CAT activity, although we detected a high concentration of an immunologically related polypeptide in MCF-7 cells with polyclonal antibodies. Western blotting showed the presence of a single protein of 62 kDa in MCF-7 cells and DNA-protein interactions with MCF-7 nuclear extract showed a strong DNA binding capacity, we have shown by UV cross-linking analyses with labeled oligonucleotides and purified factors, that only GREF1 interacts directly with DNA. Depletion of GREF1 and GREF2 by immunoadsorption and the disappearance of gel-shifted DNA-protein complexes in mobility-shift assays confirmed the specificity of the factors to interact with the palindrome. Although, the transcription factors CREB, the Jun-Fos/Fra complex of AP-1, and MLTF/USF appear to recognize related sequence elements (24, 25, 47–56), there is a marked size difference between these factors and GREF1 (80 kDa) and GREF2 (62 kDa). In human placenta extracts, the presence of protein PSE1, a dimer of 83- and 73-kDa subunits, was demonstrated. This PSE1 had immunological and sequence relationships with p70 and p86 of the Ku complex (57).

Several studies have demonstrated that protein dimers of 80–85 kDa/70–75 kDa influence transcription. Footprinting experiments showed that the proximal sequence element in the promoter of small nuclear RNA genes that influence transcription activity, was made of two subunits of 83- and 73-kDa proteins (57). The promoter region of the human collagen type IV genes was specifically recognized by the CTC factor CTCCBF, a dimer of 85 and 75 kDa subunits. This dimer has sequence homology with 80- and 70-kDa proteins of Ku and is involved in the control of divergent transcription of collagen genes (47). The Ku protein, a heterodimer of 70- and 80-kDa subunits, was first identified as a DNA-binding protein that was recog-
nized by autoantibodies from certain patients with sclero-
derma-polyarthritis (48). It is also implicated in DNA replication,
repair, and transcriptional control (59). The 72-kDa subunit is
the product of a gene family that produces different variants
of the dimer (60). In the placenta, for example, the protein dimers
are made of 80- and 62-kDa subunits. Ku, an abundant protein (4 \times 10^9 \text{copies/HeLa cell}), is mainly nuclear. In CV-1 and
LNCAP cells, no Ku-like proteins were detected. Characteristics
such as sequence homology with other transcription factors,
recognition of promoter sequences, DNA binding capacity,
dimer formation, and transcription activation capabilities sug-
gest that it could be a general regulating factor (61, 62). Several
studies also point to a possible link between Ku and the cell
cycle. A Ku80 was recently identified as a somatomatotropin recep-
tor that regulates a PP2A phosphatase. PP2A in turn, dephos-
phorylates specific GR sites (63) and is also involved in the
dephosphorylation of histone H1 in a cascade of phospho-
dephosphorylation events.

Phosphorylation of histone H1 is necessary for chromosome condensation at the M phase of the cell cycle (64), p34\text{cdc2}, the
catalytic component of the maturation/mitosis promoting fac-
tor, in complex with a regulatory cyclin protein, is a cell cycle
protein. Inappropriate synthesis of cyclins, crucial to the prop-
agation of the cell cycle, could result in the untimely stimula-
tion of cell division kinases and so lead to unwarranted cell
proliferation. The dephosphorylation of Tyr-15 and Thr-14 of
cdc2 is achieved by the specific phosphatase p80\text{cdc2} (65). By
maintaining this protein in an inactive form, PP2A inhibits its
activity and maturation/mitosis promoting factor cannot phos-
phorylate H1. Consequently, chromosome condensation does
not take place and the cell cannot proceed through mitosis. The
Ku that regulates PP2A is also a dimer of 86- and 70-kDa
subunits (66). Ku genes are activated during late G2 phase and
Ku80 dissociates from the chromosomes during mitosis (67). Ku,
also part of a DNA protein kinase, has a regulatory com-
ponent that phosphorylates RNApolII in the presence of trans-
cription factors TFIIID, TFIIB, and TFIIIF (68).

In the same manner that Ku80 is implicated in the cell cycle,
Ku70 in MCF-7 cells and GREF1 and GREF2 from placenta
could also play a role in the regulation of cell cycle kinases and
phosphatas. Many cells are unresponsive to glucocorticoids
because they lack glucocorticoid receptors (69). Inappropriate
expression of the hGR gene.

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