Characterization of promoter 1B in the human glucocorticoid receptor gene

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Abstract

At least three different promoter regions (1A, 1B, and 1C) are involved in the expression of the human GR gene. Promoters 1B and 1C are found in a 2800 bp region of DNA immediately upstream of the exon 1C transcriptional initiation site. Transcripts containing either exon 1B or 1C are expressed in a wide variety of human tissues and cultured cells. Luciferase reporter constructs were created containing promoter 1B plus 1C (−2738 to +19), promoter 1B (−2738 to −1046) alone, or promoter 1C (−1045 to +19) alone. All three constructs were equally effective in driving luciferase expression in HeLa (human cervical carcinoma) cells. In Jurkat (human T-cell acute lymphoblastic leukemia) cells, constructs containing promoters 1B plus 1C or promoter 1B were equally active, but the promoters 1B plus 1C construct was 35% more active than the promoter 1C construct. However, in HepG2 (human hepatoma) cells, the promoter 1C construct was as effective as promoters 1B plus 1C and more than twice as effective as promoter 1B. Sequences that reside proximal to the exon 1B transcriptional start site included three Sp1 (FP2-FP4) sites. Another site (FP1) contains the sequence TGATAG, which strongly resembles the consensus binding sequence for the GATA family of transcription factors. However, oligonucleotide competition and supershift analysis of FP1 indicates that this site is not a binding site for GATA proteins. These four sites are in addition to three YY1 and one Sp1 sites previously reported in promoter 1B. In HeLa cells, deletion of the three YY1 sites results in only a 30% loss of activity and substantial loss of activity occurs only after deletion of all four Sp1 sites, indicating the critical importance of Sp1 in GR expression in these cells. In contrast, the elimination of the three YY1 sites results in a dramatic decrease in promoter strength in both HepG2 and Jurkat cells (64 and 77%, respectively), while subsequent deletions of promoter elements do not result in substantial changes in promoter activity in these cell lines. This study shows that both promoters 1B and 1C are important for the ubiquitous expression of the human GR gene. Differences in the utilization of these promoters in various cell types are likely a reflection of different promoter availability and/or the levels of specific transcription factors in the cell. This could contribute to tissue-specific expression of GR levels in different cell types. © 2002 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

Glucocorticoid steroid hormones are best known for their role in the physiological response to stress, where they help an animal to adapt to unforeseen changes in their environment. While blood titers of glucocorticoids increase greatly during periods of stress, basal concentrations of corticosteroids are about 10 nM (Sapolsky et al., 2000). Given that both the steroid hormone and its cognate receptor, the glucocorticoid receptor (GR), are ubiquitous, and that the GR can interact with many other proteins including other transcription factors (Robyr et al., 2000), it is likely that one of the main functions of glucocorticoids is to maintain the GR as an active general transcription factor. While the GR is expressed in all cells studied to date, the intracellular concentration of GR protein varies greatly among cell...
types (Miller et al., 1990). The concentration of GR is an important determinant of the type and magnitude of the cellular response to the hormone (Vanderbilt et al., 1987). Consequently, processes that regulate the expression of the human GR gene are important in determining the cellular response to both basal and stress-induced levels of corticosteroids. Transcriptional regulation of the GR gene is therefore critical to the maintenance of appropriate glucocorticoid action.

Initial studies of the human GR gene characterized a promoter region associated with an untranslated exon (exon 1C) and demonstrated 11 DNase I footprints within the first 700 nucleotides upstream of the exon 1C start site (Zong et al., 1990; Nobukuni et al., 1995). Six of these footprints are binding sites for the ubiquitous transcription factors Sp1 (five sites) and AP-2 (one site) (Nobukuni et al., 1995). Deletion of the AP-2 site precipitously decreased promoter activity in HeLa and NIH3T3 cells, but not in HepG2 cells, indicating cell-type specificity of GR regulation of GR gene expression (Nobukuni et al., 1995). Deletion of one of these footprints subsequently found to be a binding site for the transcription factor Yin Yang 1 (YY1), and it may act as a promoter region associated with an untranslated exon (Zong et al., 1990; Nobukuni et al., 1995). Six of these footprints are binding sites for the ubiquitous transcription factors Sp1 (five sites) and AP-2 (one site) (Nobukuni et al., 1995). Deletion of the AP-2 site precipitously decreased promoter activity in HeLa and NIH3T3 cells, but not in HepG2 cells, indicating cell-type specificity of GR regulation of GR gene expression (Nobukuni et al., 1995). Deletion of one of these footprints subsequently found to be a binding site for the transcription factor Yin Yang 1 (YY1), and it may act as a minor start site in the human GR gene (Breslin and Vedeckis, 1998). This study also analyzed sequences distal to the transcriptional start site of the human GR promoter (−2413 to −1695) and revealed three other YY1 sites and another Sp1 site. RACE (rapid amplification of cDNA ends) analysis of human GR transcripts led to the discovery of two previously unreported human GR exons (1A and 1B) and associated promoter regions (Breslin et al., 2001). The human GR promoter 1B resides over 1000 nucleotides upstream of the exon 1C start site, while exon 1A is located approximately 31 kbp upstream of exon 1C (Breslin et al., 2001). Like promoter 1C, promoter 1B lacks a consensus TATA or CAAT box, but contains regions that are highly GC rich. The presence of multiple promoters responsible for constitutive expression of the human GR gene may underscore the critical importance of this protein to normal cellular function. However, it is clear that transcriptional regulation from the three known GR promoter regions is complex and is influenced by developmental and hormonal factors (Kalinyak et al., 1987; Breslin et al., 2001). Before we can fully understand the cellular response to glucocorticoids, a better understanding of the transcriptional regulation of the human GR gene must be obtained. To date, an analysis of the sequence proximal to the putative exon 1B transcriptional start (ca. 650 bp) has not been reported. The current study is the first to report the cell-type specific expression of promoter 1B and identifies regions within the proximal promoter 1B responsible for its usage.

2. Materials and methods

2.1. Cell culture

Human Jurkat T-cell acute lymphoblastic leukemia cells were grown in RPMI 1640 supplemented with 10% fetal bovine serum (FBS). HeLa human cervical carcinoma cells and HepG2 human hepatoma cells were grown in Eagles minimal essential medium supplemented with non-essential amino acids and 10% FBS.

2.2. Reverse transcription and polymerase chain reaction

RNA from human tissues was purchased from Clontech (Palo Alto, CA). Total RNA (2 µg) was used as template for reverse transcription (RT) using M-MuLV reverse transcriptase (NEB, Beverly, MA) and the reaction diluted 1:10 in water. Exon 1 specific sense oligonucleotides were used in combination with a human GR exon 2 specific antisense primer (Exon 2-antisense: 5'-CAGTGGATGCTGACTTGGG-3') in the polymerase chain reaction (PCR) to detect transcripts containing either exon 1B (Exon 1B-sense: 5'-GTCTGTGTGGCATTCTCAGTGCG-3') or exon 1C (Exon 1C-sense: 5'-CTTAAATATGGGCTTCC-3'). RNA quality was assessed using a human glyceraldehyde-3-phosphate dehydrogenase primer set (GAPDH-sense: 5'-GCCACATCGCTCAGACACCAT-3'; GAPDH-antisense: TCCTCAGGCTTGACGC-3'). PCR was conducted in a total volume of 20 µl using reagents from a commercial kit (Clontech Advantage 2) essentially as previously described (Breslin et al., 2001) with an initial denaturing step of 95 °C for 2 min followed by 36 cycles of 95 °C for 30 s, 60 °C for 30 s and 72 °C for 30 s. GAPDH PCR was conducted as above, except the reactions were cycled only 31 times.

2.3. DNase I protection assays

Constructs containing promoter 1B fragments were created in order to perform DNase I protection analysis. Sense (5'-TTTCTTGAGGAGCAGCCAC-3') and antisense (5'-ATTACCGGAAAGCGAGGC-3') primers were used in PCR to amplify the region of promoter 1B between −1698 and −1357. PCR products were ligated into a T/A cloning vector (pCRII; Invitrogen) and sequenced to confirm sequence fidelity. Another promoter 1B construct was created by digesting a GR promoter construct spanning −2738 to +19 (a kind gift of E. Brad Thompson, UT Medical Branch, Galveston, TX) with BstXI and SmaI restriction enzymes. The resulting fragment, spanning −1318 to −1046 of promoter 1B, was ligated into pBluescript II. Subsequently, this construct was digested with EcoRI.
and SscI restriction enzymes and the promoter 1B fragment subcloned into pGEM 2 to provide restriction sites required for Klenow labeling. DNase I footprinting was performed using HeLa nuclear protein as described previously (Breslin and Vedeckis, 1998).

2.4. Electrophoretic mobility shift assay

Four footprints were identified by DNase I footprinting using HeLa cell and NIH 3T3 (mouse fibroblast; data not shown) cell nuclear extracts. Double-stranded oligonucleotides incorporating these footprints (FP1: 5′-TCTTAGAAGGTGAGGAGCT-3′; FP2: 5′-AAGATCCGGGGTGATGGGCCCTGCACC-3′; FP3: 5′-AGTCGTAGACACATTGGCAGGGAGGGG-3′; FP4: 5′-TGGGGGTAAATGGGCAAGGCGGCGGCTCCTT-3′) were labeled and electrophoretic mobility shift assays (EMSAs) were performed as previously described (Breslin and Vedeckis, 1998). HeLa and Jurkat nuclear extracts were obtained from Geneka (Montreal, Quebec, Canada). Competition studies were carried out with the addition of 1000-fold molar excess of homologous oligonucleotide, consensus Sp1 oligonucleotide (5′-ATTCGATCGGGGCGGGGCGAGC-3′), consensus GATA oligonucleotide (5′-CAGCTGAGCTGAGGAC-3′) or unprotected promoter 1B oligonucleotide (5′-AGATCGAGGCGCAGAAAAATGTAACTCT-3′) or unprotected promoter 1C oligonucleotide (5′-AGATCGAGGCGCAGAAAAATGTAACTCT-3′) in the appropriate size were digested with appropriate restriction enzymes and subcloned into pGEM 2 to provide restriction sites required for Klenow labeling. DNase I footprinting was performed using HeLa nuclear protein as described previously (Breslin and Vedeckis, 1998).

2.5. DNA constructs

The human GR promoter fragment spanning −2738 to +19 (containing both promoters 1B plus 1C) was subcloned into the pXP-1 luciferase reporter plasmid and similar constructs containing only promoter 1B (−2738 to −1046) or promoter 1C (−1045 to +19) sequence were created as reported previously (Breslin and Vedeckis, 1998). Sense oligonucleotides, designed to incorporate a Bgl II restriction site, were used in PCR to create promoter 1B 5′ deletion constructs beginning at −1824 (1B-D1/Bgl II: 5′-AAATGAGATCT-GTTGCTGAC-3′; Bgl II site in italics), −1630 (1B-D2/Bgl II: 5′-CCAAGGAGATCTGTTAGG-3′), −1525 (1B-D3/Bgl II: 5′-AACGGAGCTGACTGTCAGGCGCAG-3′), −1322 (1B-D4/Bgl II: 5′-AAATGGCCAAGATCTCCAGCTGC-3′), −1149 (1B-D5/Bgl II: 5′-GGAGGTGAGATCTGCCTTTGC-3′) and −1115 (1B-D6/Bgl II: 5′-TCCAGTGGAGATCTCGGGCGGCG-3′) of the 1B promoter. These PCR reactions included a common antisense oligonucleotide that anneals downstream of a XmaI site present at −1046 in the human GR promoter (1B-Xma I: 5′-GTCGGGAGCTACGCTACCTT-3′). PCR products of the appropriate size were digested with Bgl II and XmaI and ligated into the same sites of pXP-1. All DNA constructs were completely sequenced to confirm fidelity and orientation of PCR products.

2.6. Luciferase assay

HeLa and HepG2 cells were plated in 6-well plates and grown to 60–80% confluence. Jurkat cells were seeded in 6-well plates at a concentration of 1 × 10⁶ cells per ml. Cells were transfected with 4 μg reporter construct (human GR promoter sequence in pXP-1) and 0.25 μg CMV/β-galactosidase normalization construct using Superfect™ (HeLa and HepG2 cells) or Effectene™ (Jurkat cells) transfection reagent (Qiagen; Valencia, CA) following the manufacturer’s instructions. Luciferase and β-galactosidase activity were assayed 48 h following transfection as previously described (Breslin and Vedeckis, 1998). Luciferase gene expression was subsequently normalized to β-gal activity. For clarity, data in Fig. 7 is expressed as a percentage of the activity of promoter 1B. The normalized data from three independent transfections were subjected to analysis of variance (ANOVA).

3. Results

3.1. Differential promoter utilization and expression of human GR exon 1 sequences

Although the GR is ubiquitously expressed, different promoters may be responsible for the expression of the GR gene in different tissues. RT-PCR was used to determine the pattern of expression of GR transcripts originating in either promoter 1B or promoter 1C. Both exon 1B and C transcripts were expressed in all human tissues examined (Fig. 1). To investigate potential intercellular differences in promoter usage, cultured human cells of different lineages were transiently transfected with human GR promoter constructs. Promoter activity was greatest in Hep G2 cells, while Jurkat cells displayed the lowest activity. In HeLa cells transfected with luciferase reporter constructs containing promoters 1B plus 1C, promoter 1B alone or promoter 1C alone, all three constructs were equally effective in driving luciferase expression (Fig. 2). In Jurkat cells, there was no statistical difference in promoter activity between the promoters 1B plus 1C construct and the promoter 1B construct alone. However, there was a significant difference (P < 0.01) between the promoter activities of the promoters 1B plus 1C construct compared with the promoter containing only promoter 1C.
3.2. Identification of four additional footprinted sites within promoter 1B

DNase I footprinting was employed to identify regions of promoter 1B that might act as binding sites for proteins involved in transcriptional regulation. Four footprints corresponding to three YY1 and a Sp1 site were previously identified in promoter 1B (Breslin and Vedeckis, 1998). Four additional footprints were identified in the current study using DNase I protection with HeLa nuclear extract (Fig. 3 and Fig. 4). The protected sequences were analyzed using SIGNAL SCAN (Prestridge, 1991) (http://bimas.dctr.nih.gov:80/molbio/signal/) and PROMOTER SCAN programs (Prestridge, 1995) (http://biosci.cbs.umn.edu/software/proscan/promoterscan.htm). Footprint 1 (FP1) (corresponding with −1564 to −1549 of promoter 1B) resembled the consensus binding sequence for the GATA family of transcription factors (WGATAR). The other three footprints (FP2-FP4; corresponding with −1510 to −1498, −1275 to −1258, and −1251 to −1231, respectively) were GC rich and resembled binding sites for the ubiquitous transcription factor Sp1.

EMSA was used to provide more definitive information about the protein factors responsible for the DNase footprints. The addition of labeled double-stranded oligonucleotides incorporating FP1 to HeLa cell nuclear extract resulted in two distinct complexes (Fig. 5A). The addition of 1000X cold FP1 oligonucleotide effectively competed out the binding of labeled...
FP1, but 1000X unprotected promoter 1B sequence that resides between FP1 and FP2 (Fig. 4) had no effect. Mutating the sequence resembling the GATA consensus site to a sequence known to disrupt GATA binding (TGATAG to TCTTAG) abolished protein binding and 1000X excess of this mutant oligonucleotide failed to compete with labeled wild-type FP1 (data not shown). However, the addition of 1000X cold GATA consensus oligonucleotide also did not compete for FP1 binding, and the addition of 2 μg of anti-GATA-3 antibody failed to alter the FP1/protein complexes (Fig. 5A). Four complexes were formed in a similar experiment using Jurkat cell nuclear extract (Fig. 5B). The addition of 1000X excess mutant FP1 oligonucleotide corresponding to the preferred GATA binding consensus sequence (TGATAG to TGATAA) competed as well as 1000X of wild-type oligonucleotide. However, as with HeLa cell nuclear extract, GATA consensus oligonucleotide (1000X) failed to compete for protein binding and anti-GATA-3 antibody failed to alter the FP1/protein complex. Therefore, the protein responsible for FP1 in promoter 1B does not appear to be a member of the GATA family of transcription factors, and the protein(s) responsible for this footprint remain unidentified.

EMSA was also used to characterize proteins responsible for the remaining three footprints. Labeled double-stranded oligonucleotides incorporating either FP2, FP3, or FP4 were shifted by HeLa cell nuclear extract, and the addition of 1000X cold homologous oligonucleotide effectively competed for binding (Fig. 6A). In all cases, the addition of 1000X unprotected promoter 1B oligonucleotide (−1536 to −1515) did not compete for binding. The addition of 1000X cold Sp1 consensus oligonucleotide effectively competed for binding, and addition of 2 μg of anti-Sp1 antibody to the EMSA reactions further retarded the migration of the oligonucleotide/protein complexes through the gel. Moreover, the addition of 1000X cold FP2, FP3, or FP4 effectively competed out labeled Sp1 consensus oligonucleotide, while unprotected promoter 1B sequence had little effect (Fig. 6B). These data indicate that FP2, FP3, and FP4 are binding sites for the ubiquitous transcription factor Sp1.

3.3. Deletion analysis of promoter 1B

To determine the contribution of promoter 1B footprints to promoter strength, a deletion series of promoter 1B/luciferase constructs was created. Sequential 5′ deletions of promoter 1B revealed cell-type specific differences in the importance of the distal promoter regions (Fig. 7). In transiently transfected Jurkat and HepG2 cells, deletion of the region between −2738 and −1824 of promoter 1B (containing the three YY1 sites) reduced promoter activity by 77% (P < 0.001) and 64% (P < 0.001), respectively. In transiently transfected HeLa cells, the deletion of the YY1 sites reduced promoter activity by only 30% (P < 0.01). Further deletion of promoter 1B did not result in substantial differences in promoter activity between the −1824 to −1046, −1630 to −1046, −1525 to −1046 or −1322 to −1046 constructs in any cell type examined, although all constructs had significantly higher promoter activity than the promoterless pXP-1 vector (P < 0.001). However, the activities of the −1149 to −1046 and −1115 to −1046 constructs were indistinguishable from the promoterless pXP-1 vector. These results indicate that the majority of promoter 1B activity resides between −1824 and −1149 in HeLa cells, while YY1 may drive the majority of promoter 1B activity in Jurkat and HepG2 cells.

4. Discussion

Human GR transcripts have been detected in all cell types studied till date. However, the intracellular concentration of human GR protein is not constant among cell types. As the cellular response to glucocorticoids is in large part due to the concentration of GR, it is imperative that GR expression be strictly regulated. At least three different promoters are responsible for the expression of the human GR gene (Breslin et al., 2001).
The human 1A promoter may contain glucocorticoid-response elements necessary for hormone-mediated up-regulation by GC in T-cells and down-regulation in other cell types. The 1B and 1C promoters are most likely responsible for constitutive expression of the human GR. Transcripts containing exon 1B and 1C are coexpressed in human brain, cerebellum, spinal cord, heart, trachea, lung, kidney, liver and placenta. Our previous study detected exon 1B and 1C specific transcripts in Jurkat, HeLa S3, HepG2, IM-9 (human B-cell lymphoma), CEM-C7 (human T-cell acute lymphoblastic leukemia), HL-60 (acute myeloid leukemia), MCF-7 (breast carcinoma), 786-0 (kidney carcinoma), SISA (osteosarcoma), H1299 (lung carcinoma) and WI-38 (normal human fibroblasts) cells (Breslin et al., 2001). As shown in the current study, although both 1B and 1C transcripts are coexpressed in all cells examined, there appear to be cell-specific differences in GR promoter activity. In HeLa cells, there was no significant difference in promoter activity between constructs containing promoter 1B plus 1C, promoter 1B alone, or promoter 1C alone. Jurkat cells displayed the least activity of the three cell lines assayed; indeed, a substantial amount of GR transcripts in hematopoietic cell

Fig. 4. DNase I footprinted sites within human GR promoter 1B. Shown is the proximal human GR promoter 1B and associated exon 1B sequence. A further 900 bp upstream was previously characterized and a Sp1 site identified by this study is underlined (Breslin and Vedeckis, 1998). The positions at which deletion constructs begin (see Fig. 7) are marked by bold arrows and numbered D1 through D6. Sequences of oligonucleotides containing DNase I footprints (bold) are boxed. The double-underlined nucleotides are the 5' ends of 11 cloned 5' RACE products obtained using a primer in exon 2 (M.B. Breslin and W.V. Vedeckis, unpublished). The putative cap site based on the 5' RACE ends and computer analyses is also indicated by an arrow and labeled 'CAP'. The exon 1B/exon 2 splice site is indicated by an open triangle. The italicized sequence is the 'unprotected promoter 1B' oligonucleotide that was used as a control in Figs. 5 and 6.
lines appear to originate from promoter 1A, with lesser amounts of transcripts originating from promoters 1B or 1C (Breslin et al., 2001). In Jurkat cells there was no significant difference between the constructs containing promoters 1B plus 1C and promoter 1B alone, but both were more active than the construct containing the promoter 1C alone. In HepG2 hepatocarcinoma cells, promoter 1C appears to contribute substantially more promoter activity than promoter 1B. As HepG2 cells retain many of the differentiated characteristics of normal hepatocytes, it is not surprising that this cell line displays the highest activity of the three cell lines studied. Differences in promoter usage among these three distinct cell lines imply distinct cell-specific regulation and roles for these promoters.

Constitutive or ‘housekeeping’ genes typically lack a TATA or CAAT box, but contain multiple GC rich regions that are potential binding sites for ubiquitous transcription factors such as Sp1. Previous studies have identified five Sp1 sites within promoter 1C, and a single Sp1 site in promoter 1B (Nobukuni et al., 1995; Breslin and Vedeckis, 1998). The identification of three additional Sp1 sites within promoter 1B is further proof that this transcription factor and promoter are important for the universal constitutive expression of the GR. Promoter 1C contains an AP-2 site, also common to ubiquitously expressed genes, and an AP-1 site within promoter 1C may be involved in the regulation of the GR gene (Wei and Vedeckis, 1997). Active AP-1 or AP-2 sites within promoter 1B have not been identified. Both promoters 1B (3 sites) and 1C (1 site) contain YY1 binding sites that are important for GR expression (Breslin and Vedeckis, 1998). YY1, expressed in a wide variety of mammalian cell types, is a zinc-finger transcription factor that can act as an activator, a repressor, or an initiator of transcription (Shrivastava and Calame, 1994). Deletion of the four YY1 sites from the intact −2738 to +19 GR promoter sequence elicited a 60% decrease in promoter activity in HeLa cells (Breslin and Vedeckis, 1998). However, when examining the activity of the isolated 1B promoter, removing the YY1 sites resulted in only a 30% decrease in promoter activity in HeLa cells. This may indicate that other sites in the GR promoter 1B, most likely the multiple Sp1 sites, are more important in driving GR expression in HeLa cells. When Sp1 sites are deleted, the remaining Sp1 sites appear to be sufficient in preventing any appreciable loss of promoter activity. This thought is bolstered by the fact that serial deletion of promoter 1B sequence does not appreciably alter promoter activity in HeLa cells until the loss of the final two Sp1 sites. In Jurkat and HepG2 cells, it is apparent that the YY1 sites are relatively more important than in the HeLa cells, as removal of these sites results in significant reductions in promoter activity. Further loss of promoter activity in these cells does not occur until the final two Sp1 sites are deleted. Recent, preliminary experiments indicate that there may be an additional GR promoter that may result in transcripts that initiate near the three upstream YY1 sites (B.S. Nunez and W.V. Vedeckis, unpublished observation).

While the identification of multiple Sp1 and YY1 sites within promoter 1B may underscore its constitutive nature, other processes may act to regulate GR expression from promoters 1B and 1C. FP1 is an intriguing site, given its striking resemblance to the GATA binding sequence. The GATA family of transcription factors is critical to the development of many...
hematopoetic lineages. Both HeLa and Jurkat cells are known to express GATA proteins (Dorfman et al., 1992; Penix et al., 1993), yet consensus GATA oligonucleotides do not compete for FP1 binding. Although Jurkat cells are known to express GATA-3 (Penix et al., 1993), the addition of GATA-3 antibody does not alter FP1 binding to proteins from Jurkat cell nuclear extract. Thus, in spite of the similarity in the sequence of FP1 and GATA sites, the protein(s) responsible for FP1 does not appear to belong to the GATA family. The identity of this protein(s) has not yet been discovered, but the importance of FP1 in the cell types studied is uncertain due to the fact that its removal from promoter constructs does not alter promoter activity. It is possible that this site is more important in other cell types or becomes more important in other, as-yet-undefined, conditions in the cell types studied.

While the simple expression of GR is probably critical to general cellular function (e.g. carbohydrate and protein metabolism), it is perhaps more important that transcriptional regulation of the human GR gene be sufficiently flexible to allow a single cell to respond to glucocorticoids in a manner which best suits its cellular function, including those that are highly specialized. It is clear that promoters 1B and 1C are responsible for constitutive expression of the human GR, but that expression from these promoters is complex and driven by a variety of transcription factors. This study has provided evidence that promoter 1B can act on its own (that is, independently of promoter 1C) and serves as a constitutive promoter. However, it is evident that the expression from this promoter can vary among cell types, and this may be important in the differential sensitivity of various genes to glucocorticoids in different cell types.

Fig. 6. EMSA analysis of FP2, FP3, and FP4 in human GR promoter 1B. Double stranded oligonucleotides incorporating either FP2, FP3 or FP4 (corresponding with the sequence indicated in Fig. 4) were labeled, mixed with HeLa nuclear extract and resolved using native PAGE (Panel A). Other reactions included 1000X cold homologous oligonucleotide, 1000X unprotected promoter 1B oligonucleotide, 1000X cold Sp1 consensus oligonucleotide, or 2 μg of an antibody developed to human Sp1. Similarly, a double stranded oligonucleotide incorporating the consensus Sp1 binding site was labeled, mixed with HeLa cell nuclear extract and resolved using native PAGE (panel B). Other reactions included either 1000X cold Sp1 consensus oligonucleotide, 1000X unprotected promoter 1B oligonucleotide, 1000X FP2, 1000X FP3, 1000X FP4 or 2 μg of an antibody developed to human Sp1.
Fig. 7. Deletion analysis of human GR promoter 1B. Deletion constructs of promoter 1B were constructed as described in Section 2 and as indicated in Fig. 3. Positions of known transcription factor binding sites and putative transcription start site within the constructs are as indicated. Promoter 1B/pXP1 luciferase reporter constructs were cotransfected with a CMV/β-galactosidase normalization reporter construct into Jurkat (solid bars), HepG2 (hatched bars) or HeLa (open bars) cells. Cells were collected 48 h after transfection and luciferase and β-gal activity was measured in the cell lysate. Luciferase activity was normalized to β-gal activity and subjected to analysis of variance. For clarity, data are presented as percentage of the activity of the −2738 to −1046 promoter 1B construct. Error bars indicate S.E.M. of three independent transfections and asterisks indicate a significant difference from the full-length promoter 1B construct (*, P < 0.05; **, P < 0.01; ***, P < 0.001).

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