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Cloning and characterization of hGMEB1, a novel glucocorticoid modulatory element binding protein

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Abstract A 21-bp element called glucocorticoid modulatory element (GME) modulates the glucocorticoid receptor-mediated responses via the binding of an as yet poorly characterized *trans*-acting complex of proteins containing the 88-kDa GMEB1 and the 67-kDa GMEB2. Using heat shock protein 27 (HSP27) as bait in the yeast two-hybrid assay, we cloned a 1.83-kb cDNA encoding a novel 573-amino acid protein called human GMEB1 (hGMEB1). hGMEB1 possesses a KDWK domain, contains sequences almost identical (36/38) to three tryptic peptides of rat GMEB1 and shares 38% identity with rat GMEB2. hGMEB1 is ubiquitously expressed as a 85-kDa protein in all cell lines and tissues examined. In vitro translated hGMEB1 bound specifically to GME oligonucleotides yielding a complex of similar size to the complex obtained using rat liver nuclear extracts. Both complexes were supershifted with an antibody specific to hGMEB1. Co-immunoprecipitation experiments confirmed the in vivo interaction of HSP27 with hGMEB1.

Key words: Transcription factor; HSP27; Glucocorticoid modulatory element binding protein

1. Introduction

The tyrosine aminotransferase (TAT) gene possesses a number of *cis*-acting elements allowing liver tissue-specific regulation by multiple converging signaling pathways such as those activated by glucocorticoids, insulin and activators of protein kinase A and protein kinase C. Glucocorticoid tissue-specific regulation occurs in part through the cooperative action of a glucocorticoid response element (GRE) and a binding site for the liver-specific hepatocyte nuclear factor-3 located in a glucocorticoid-responsive unit at between −2.6 and −2.3 kb of the start codon [1–3]. In unstimulated cells, the glucocorticoid receptor is found in the cytoplasm associated with a complex of heat shock proteins composed of HSP90, HSP70 and p60 [4]. Upon binding to glucocorticoids, the receptor translocates into the nucleus where it forms DNA-receptor-steroid complexes at the GRE resulting, through interactions with coactivators, in an enhanced transcriptional activity [5,6].

A 26-bp cAMP response element (CRE) together with a hepatocyte nuclear factor-4 binding site situated 50 bp apart from each other at −3.6 kb from the start site provides the liver-specific cAMP-dependent transcriptional activation of the TAT gene and is the site of inhibition of TAT induction by phorbol esters (protein kinase C pathway) and insulin [2,7–11]. TAT-CRE is an asymmetrical and low affinity CRE, which has low enhancer activity by itself [8]. A 6× concatenated repeat of TAT-CRE or the presence of a hepatocyte nuclear factor-4 binding site in tandem with a single repeat of TAT-CRE is required to confer cAMP responsiveness to a minimal heterologous promoter [2]. However, another activity has been ascribed to this element. A 21-bp oligonucleotide overlapping with the CRE and named glucocorticoid modulatory element (GME) causes a cell type- and cell density-dependent left shift in the dose-response curve of sub-saturating concentrations of glucocorticoids and enhances the gene response to saturating concentrations of anti-glucocorticoids (e.g. dexamethasone 21-mesylate) [12–17]. In tandem with a GRE in a minimal promoter gene, GME does not increase the maximum response to glucocorticoid but alters the EC_{50} of the gene dose-response curve [13,14]. Gel shift assays using TAT-CRE/GME oligonucleotides identified three protein complexes. Two of these complexes are competed by an authentic symmetric CRE and have been shown to contain the cAMP response element binding protein (CREB) [7,8]. CREB is a member of the CREB/CREM/ATF-1 family of transcriptional factors that requires phosphorylation mediated predominantly by protein kinase A for full activity [18]. The other protein complex is not competed by symmetric CRE. It corresponds to a multimeric complex of some 550 kDa, composed of two proteins of 88 and 67 kDa denoted GME binding protein-1 (GMEB1) and GMEB2, respectively [19]. Rat GMEB2 is unrelated to CREB and belongs to a new family of KDWK domain-containing nuclear factors [20]. From the partial amino acid sequences of three tryptic fragments, rat GMEB1 also appears to be a new protein unrelated to CREB [19].

In the present study, we report the isolation of a cDNA encoding a novel KDWK domain-containing protein 38% identical to rat GMEB2 and with sequences 95% identical to the three tryptic peptides of rat GMEB1. In the gel shift assay, the in vitro translated protein called hGMEB1 displaced radiolabeled TAT-CRE/GME to the same level as rat liver nuclear extract. In both cases, the complexes were supershifted by a polyclonal antibody reacting specifically against hGMEB1. It is thus concluded that hGMEB1 is a novel *trans*-acting DNA binding protein that may mediate the demonstrated functions of GME as a positive modulator of the glucocorticoid response.

2. Materials and methods

2.1. Cell culture

HeLa, CCL39 and 293 cells were grown in DMEM medium supplemented with 10% fetal bovine serum. CHO cells were grown in α-MEM medium with 10% fetal bovine serum. NIH 3T3 cells were grown in DMEM medium supplemented with 10% calf serum. Hu-

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man umbilical vein endothelial cells (HUVEC) were established and grown as described [21,22].

2.2. Buffers

PBS contained 137 mM NaCl, 5 mM KCl, 10 mM Na_2HPO_4 and 11 mM glucose, pH 7.2. TNEPT buffer was composed of 20 mM Tris-HCl, pH 7.4, 120 mM NaCl, 1 mM EDTA, 0.1 mM $NaVO_4$, 1 mM phenylmethylsulfonyl fluoride and 1% Triton X-100. The gel shift assay buffer was made of 10 mM HEPES/KOH pH 7.9, 4% Ficoll 400, 30 mM KCl, 5 mM $MgCl_2$, 4 mM spermidine, 0.1 mM EDTA pH 8.0, 0.25 mM DTT and 0.25 mM phenylmethylsulfonyl fluoride. TBS was made of 10 mM Tris-HCl pH 7.4 and 150 mM NaCl.

2.3. Two-hybrid screening

Full-length hamster HSP27 was cloned in phase with LexA in pBTM116 vector to produce a bait fusion protein [23]. The library used for screening was a HeLa cDNA library cloned at the *Eco*RI-*Xho*I site of pGAD GH (Clontech). Two-hybrid screening was done by sequential transformation of bait and library vectors in L40 yeast [23,24]. After transformation, yeasts were plated on triple-selective media (lacking histidine, leucine and tryptophan) and incubated at 30°C until colonies appeared. Colonies that grew more than 2 mm were tested for β-galactosidase activity using a colony lift filter assay. The plasmids from positive clones were purified, co-transfected with LexA-Ras(V12) to identified false-positive clones [25] and then probed by Southern blot with a HSP27 cDNA probe to eliminate HSP27 clones. Among the remaining positive clones, clone 70-1 was picked as the one yielding the strongest signal and used to identify by Southern blot a number of related plasmids including clone 130-5. The *Eco*RI-*Xho*I inserts of clone 130-5 and 70-1 were introduced at the *Eco*RI-*Xho*I site of pGEX-4T-3 to produce plasmids pGEX-4T-3-hGMEB1 and pGEX-4T-3-hGMEB1/CT, respectively. The plasmids were used to produce the glutathione *S*-transferase (GST) fusion proteins GST-hGMEB1 and GST-hGMEB1/CT in the *Escherichia coli* protein expression system of Pharmacia (Uppsala, Sweden) according to the manufacturer's instructions. The *Eco*RI-*Xho*I fragment of clone 130-5 was also inserted at the *Eco*RI-*Sal*I site of the expression vector pCMV5 to produce plasmids pCMV5-hGMEB1. pCIneo-myc-hGMEB1 was constructed in pCIneo (Promega, Madison, WI, USA) by inserting a double-stranded oligonucleotide corresponding to the myc epitope in frame with the open reading frame contained in clone 130-5.

2.4. Immunodetection of GMEB1

The polyclonal antibody EL-71 recognizing hGMEB1 was raised in rabbit against the fusion protein GST-hGMEB1/CT. Cell and mouse tissue extracts were prepared in SDS-PAGE sample buffer. The proteins were separated by electrophoresis, transferred on nitrocellulose membranes, and probed with EL-71 diluted 1/1000 in TBS. Antigen-antibody complexes were revealed with HRP-anti-rabbit IgG antibodies diluted 1/5000 in TBS using the ECL detection kit (Amersham).

2.5. Immunofluorescence microscopy of hGMEB1

Immunofluorescence was performed on HeLa cells growing on glass coverslips. Control cells or cells transfected with pCIneo-myc-hGMEB1 were fixed with 3.7% formaldehyde for 10 min and permeabilized with 0.1% Triton X-100 for 1 min at room temperature. hGMEB1 was detected using the EL-71 antibody diluted 1/50 in PBS and the hGMEB1 antigen-antibody complexes were revealed with FITC-labeled anti-rabbit IgG antibodies diluted 1/25 in PBS. myc-hGMEB1 was detected using the anti-myc antibody 9E10 diluted 1/100 in PBS and the antigen-antibody complexes were revealed with Texas red-labeled anti-mouse IgG diluted 1/50 in PBS. Confocal microscopy was performed using a Bio-Rad MRC-1024 imaging system mounted on a Nikon Diaphot-TDM equipped with a 60× oil objective lens with a numerical aperture of 1.4.

2.6. Coimmunoprecipitation and GST pull-down assay

Five μg of the expression vector pCMV5-hGMEB1 and pCIneo-huHSP27 were transfected alone or in combination in exponentially growing HeLa or NIH 3T3 cells (4×10^5 cells in 25-cm^2 tissue culture flasks) by calcium phosphate precipitation. The empty plasmids pCMV5 or pCIneo were used as filling DNA to bring the final plasmid concentration to 10 μg DNA per flask. After 48 h, the cells were lysed in TNEPT buffer, centrifuged at $17\,000\times g$ for 10 min at 4°C and processed either for immunoprecipitation or for GST pull-down assays. For immunoprecipitation, the clarified supernatants were incubated for 45 min at 4°C in TNEPT buffer with anti-human HSP27 (Hu27) absorbed to protein A-Sepharose. GST pull-down was done similarly except that the extracts were incubated with GST, GST-hGMEB1 or GST-hGMEB1/CT coupled to glutathione beads. In both cases, the beads were pelleted and washed four times in TNEPT buffer. The pellets as well as a portion of the total cell extracts were processed for immunodetection with either anti-hGMEB1 or anti-HSP27.

2.7. Electrophoretic mobility shift assay

Rat liver nuclear extracts were prepared as reported [26]. Primed (hGMEB1-producing) and unprimed reticulocyte lysates were prepared using the plasmids pCIneo-myc-hGMEB1 and empty pCIneo, respectively, in the TNT T7 rabbit reticulocyte lysate expression system of Promega (Madison, WI, USA), according to the manufacturer's protocol. A ^{32}P-labeled doubled-stranded GME oligonucleotide sequence corresponding to the rat TAT sequence at position between −3654 and −3634 bp from the start of transcription was produced by annealing the oligonucleotides 5′-gatctCTTCTGCGTCAGCGCCAG-TATg-3′ and 3′-aGAAGACGCAGTCGCGGTCATAcctag-5′ (lower-case letters indicate added nucleotides to produce *Bgl*II and *Bam*HI cohesive ends) and filling the 5′ protruding ends with Klenow enzyme in the presence of [α-^{32}P]dATP (Dupont Canada Inc.) at room temperature. The oligonucleotide was purified over a 12% polyacrylamide gel and electroeluted for 45 min at room temperature. ^{32}P end-labeled GME probes (10.5 fmol, 180 000 cpm) were incubated with adult rat liver nuclear extract (3 μg) or reticulocyte lysates (2 or 4 μl) in a total volume of 10 μl for 30 min at room temperature with, in addition, boiled and autoclaved salmon sperm DNA (0.3 μg) and 20× single-stranded unlabeled GME. Competition experiments used the following unlabeled and unfilled double-stranded oligonucleotides (only the upper strand sequence is given): TAT GME, see above; consensus GRE, 5′-gatctTGTACAGGATGTTCTg-3′ (lower-case letters: *Bam*HI and *Bgl*II sites); collagenase AP1, 5′-aattcGTGTCTGACT-CATGCTTTg-3′ (*Eco*RI sites); and symmetric CRE consensus, 5′-aattcAAATTGACGTCATGGTAAg-3′ (*Eco*RI sites). In supershift experiments, the antibodies were added with the protein extracts 30 min at room temperature before addition of the labeled probe. Electrophoresis was carried out in 5% non-denaturing polyacrylamide gel at 150 V in 0.25× Tris-borate-EDTA buffer. The dried gels were autoradiographed for 8–15 h at room temperature with Kodak X-omat XAR-5 film.

3. Results

3.1. Cloning and sequence analysis of hGMEB1

A two-hybrid screen of a plasmid library of fusions between the activation domain of Gal4 and poly-dT-amplified cDNA from HeLa cells was made using full-length Chinese hamster HSP27 fused to the LexA DNA binding domain as bait. Among the clones obtained that were confirmed to produce real HSP27 interacting proteins, one clone (clone 70-1) contained a 461-bp sequence encoding a 115-amino acid peptide (Fig. 1). Southern blot analysis of the other clones using clone 70-1 as a probe identified 28 clones of a length similar to 70-1 and four longer clones. The longer species, typified by clone 130-5, were redundant clones of a 1828-bp cDNA containing an open reading frame of 573 amino acids starting at position 7. Clone 70-1 corresponded to the 3′ end of clone 130-5 and encoded the last 115 amino acids of the protein.

Search for homology with published sequences using the BLAST program [27] revealed 38% identity between the peptide encoded by clone 130-5 and the 67-kDa rat glucocorticoid modulatory element binding protein GMEB2 (Fig. 1A). The search also identified a region located between residues 92 and 185, having a high degree of similarity with a number of proteins in a domain called KDWK [28]. Rat GMEB2 and clone 130-5 peptide are 80% identical in the KDWK domain

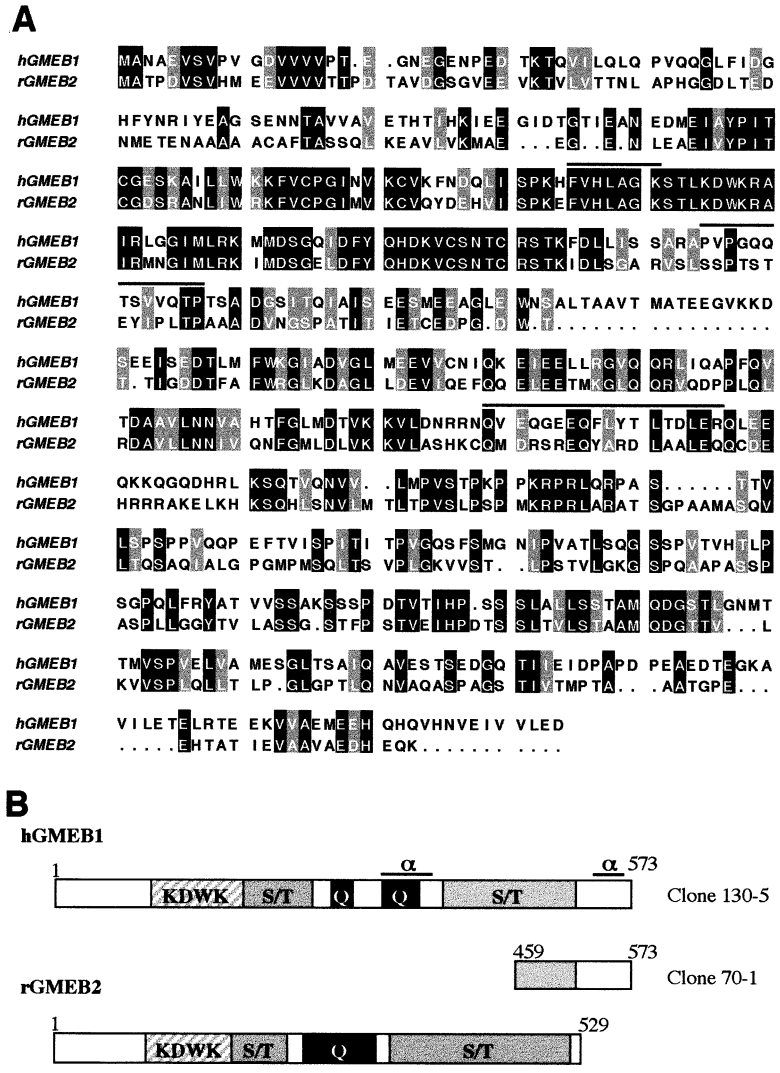

Fig. 1. A: Amino acid sequence comparison of hGMEB1 with rat GMEB2. Alignment of deduced amino acid sequences of clone 130-5 (hGMEB1) with rat GMEB2 (rGMEB2 [20], GenBank accession number AF059273). Black and gray backgrounds indicate identity and similarity, respectively. Overlined sequences identify three partially sequenced tryptic fragments of rat GMEB1 [19]. The nucleotide and deduced amino acid sequences of hGMEB1 have been deposited in the GenBank database under GenBank accession number AF099013. B: Schematic representation of the protein encoded by hGMEB1 clone 130-5, clone 70-1 and rat GMEB2. Hatched boxes refer to the conserved KDWK domain. S/T and Q indicate serine/threonine-rich and glutamine-rich regions, respectively. α indicates putative α-helix coiled-coil domains as predicted by MacStripe [48].

(Fig. 2). Rat GMEB2 binds TAT-CRE/GME as a multimeric complex with an 88-kDa protein called rat GMEB1 [20]. Rat GMEB1 has been purified to homogeneity and the sequences of three tryptic peptides have been determined [19]. Two of these peptides were found integrally in clone 130-5; 16 of the 18 residues of the third peptide are conserved (overlined sequences in Fig. 1). This important similarity of sequence with rat GMEB2 and particularly with the tryptic fragments of rat GMEB1 suggested that the protein encoded by clone 130-5 was a human GMEB and probably the human GMEB1. For these reasons, the protein was named hGMEB1. Other features of hGMEB1 sequences are summarized in Fig. 1B and described in Section 4. The nucleotide and deduced amino acid sequences of hGMEB1 have been deposited in the GenBank database under GenBank accession number AF099013.

3.2. hGMEB1 is a ubiquitously expressed nuclear protein

An antibody against hGMEB1 (EL-71) was prepared in rabbit using a recombinant fusion protein made of GST and the last 115 amino acids of hGMEB1 (hGMEB1/CT). In Western blot using EL-71, a polypeptide of some 85 kDa was recognized in a number of cell lines from human (HeLa, 293 and HUVEC), Chinese hamster (CCL39, CHO) and mouse (NIH 3T3), in rat liver and different mouse tissue extracts (Fig. 3A). The apparent molecular weight of the EL-71-reacting polypeptides contrasted with the predicted molecular weight of 62.5 kDa calculated from the protein sequence of hGMEB1 and might reflect the high content of acidic residues in hGMEB1. A band with identical mobility was strongly expressed in extracts from HeLa cells that were transfected with pCMV5-hGMEB1. This result and the fact that the N-terminus sequences of hGMEB1 and rat GMEB2 were highly conserved (Fig. 1A) suggested that clone 130-5 contained the entire coding sequence of hGMEB1. The EL-71 antibody also detected a smaller product migrating at 43 kDa. The 43-kDa species was particularly strong in tissue

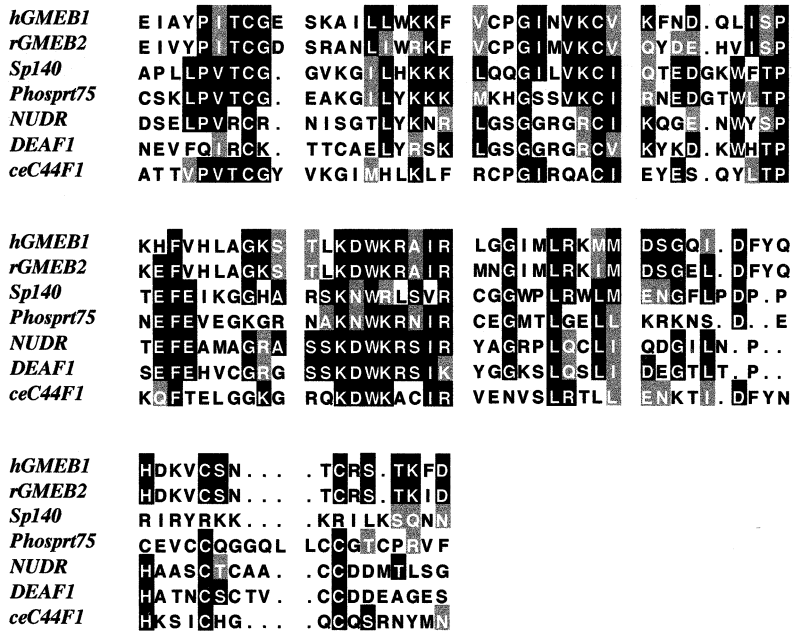

Fig. 2. Alignment of the KDWK domain of hGMEB1 and other proteins. Alignment of KDWK domain of different proteins: human GMEB1, rat GMEB2 (GenBank accession number AF059273), human Sp140 protein (GenBank accession number U63420), human phosphoprotein 75 (Phosphoprt75, GenBank accession number L22343), human NUDR (GenBank accession number AF049459), *Drosophila* DEAF1 (GenBank accession number AF025458) and open reading frame from *C. elegans* (GenBank accession number Z49067). Amino acids identical or similar in four or more proteins are printed over a dark or gray background, respectively.

extracts suggesting that it could be a degradation product of hGMEB1.

The intracellular localization of hGMEB1 was determined by immunofluorescence in control HeLa cells and in HeLa cells transfected with pCIneo-myc-hGMEB1. Both the endogenous hGMEB1 detected with the EL-71 antibody and the transfected myc-tagged proteins detected by either the EL-71 or the 9E10 antibodies showed a strong nuclear localization (Fig. 3B).

3.3. hGMEB1 interacts with the GME oligonucleotide

To confirm that hGMEB1 was a GMEB protein, myc-tagged hGMEB1 (myc-hGMEB1) was translated in a reticulocyte lysate and tested in gel shift assay using ^{32}P-labeled GME. In contrast to unprogrammed reticulocyte lysates (empty vector) which yielded no complex formation, lysates programmed with myc-hGMEB1 cDNA produced a discrete gel-shifted band (Fig. 4A, lanes 1 and 2). The band was further retarded by 9E10 anti-myc, EL-71 anti-hGMEB1 but not by 12ca5 anti-hemagglutinin (an irrelevant antibody) or preimmune serum (Fig. 4A, lanes 9–12). The complex was specific, being competed by excess cold GME but not by GRE, CRE or AP-1 oligonucleotides (Fig. 4A, lanes 3–8 and Fig. 4B, lanes 3–5). Rat liver nuclear extracts produced a more complex pattern of gel-shifted complexes (Fig. 4C), in agreement with previous studies indicating that CREB in addition to GMEB can bind GME [7,8]. All complexes were competed by unlabeled GME; however, only the slowest migrating

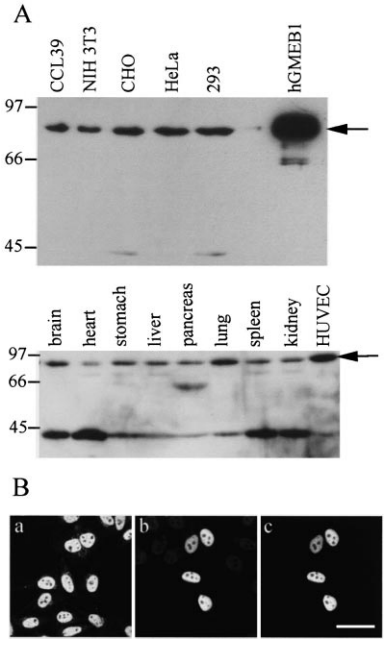

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Fig. 3. Expression and intracellular localization of GMEB1 protein. A: Cell extracts were prepared from Chinese hamster CCL39 and CHO (80 μg), mouse NIH 3T3 (80 μg), human HeLa and 293 (80 μg), HUVEC (40 μg), HeLa cells transfected with pCMV5-hGMEB1 (hGMEB1, 9 μg) or from various mouse tissues as indicated (40 μg). The proteins were separated by SDS-PAGE and transferred on a nitrocellulose membrane for Western analysis. The arrows indicate the position of hGMEB1 as detected using EL-71. Numbers on the left indicate the position of molecular weight markers. B: Control HeLa cells (a) or HeLa cells transfected with pCIneo-myc-hGMEB1 (b and c) were fixed and processed for immunofluorescence microscopy using anti-hGMEB1 EL-71 antibody (a and b) or anti-myc 9E10 antibody (c). b and c are from the same field using FITC and Texas red-labeled anti-rabbit and anti-mouse secondary antibodies respectively. Note that endogenous hGMEB1 is barely visible in b due to the use of a lower intensity scale.

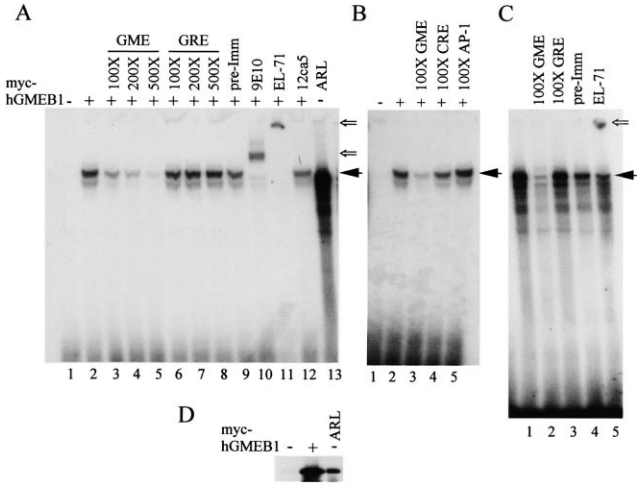

Fig. 4. Binding of hGMEB1 to GME oligonucleotide in gel shift assay. Protein-DNA complexes were separated by non-denaturing electrophoresis after incubating a ^{32}P-labeled GME oligo with 2 μl (B) or 4 μl (A) of unprogrammed (−) or myc-hGMEB1 programmed (+) reticulocyte lysates, or 3 μg of adult rat liver extract (ARL; lane 13 in A, and lanes 1–5 in C). Binding was competed with the indicated (100, 200 or 500×) molar excess concentrations of unlabeled GME, GRE, CRE or AP-1 oligos. Supershift assays were performed using a preimmune serum (pre-Imm), anti-hGMEB1 (EL-71), anti-myc (9E10) and anti-hemagglutinin (12ca5). The filled arrows indicate the GME/GMEB1 complex. The open arrows indicate the position the antibody-shift complexes. In D, Western blot of hGMEB1 in unprogrammed (−), programmed lysate (+) (10 μl each) and ARL (15 μg) using anti-hGMEB1 (EL-71).

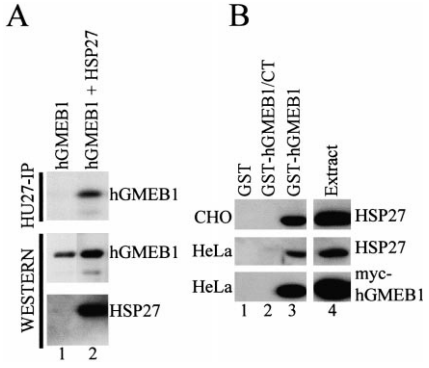

Fig. 5. hGMEB1 and HSP27 interact with each other in mammalian cells. A: Co-immunoprecipitation. NIH 3T3 cells transfected with pCMV5-hGMEB1 (lane 1) or with pCMV5-hGMEB1 and pCIneoHSP27. Total extracts were analyzed by Western blot (Western) for expression of hGMEB1 and HSP27, or immunoprecipitated with anti-HSP27 (HU27-IP) and tested for hGMEB1 co-immunoprecipitation. B: GST pull-down assay. Extracts from CHO cells, HeLa cells or pCIneo-myc-hGMEB1 transfected HeLa cells were incubated with immobilized GST, GST-hGMEB1 or GST-hGMEB1/CT. Bound proteins were analyzed by Western blot for the presence of HSP27 and myc-hGMEB1 using Hu27 and 9E10 antibodies, respectively. A fraction of the extracts (5% for HSP27, 10% for myc-hGMEB1) was also analyzed directly by Western blot to determine the amount of total HSP27 or myc-hGMEB1 in the cell extracts (Extract).

band, which migrated at a rate comparable to the complex formed with myc-hGMEB1 produced in reticulocyte lysates, was supershifted by EL-71 (Fig. 4C, lane 5).

3.4. Interaction of hGMEB1 and HSP27

Further analyses of the interaction of HSP27 with hGMEB1 in yeast confirmed that HSP27 interacted with both hGMEB1 and hGMEB1/CT, albeit six times more β-galactosidase activities were generated with hGMEB1 as compared to hGMEB1/CT (data not shown). To determine whether hGMEB1 interacts with HSP27 in mammalian cells, NIH 3T3 cells were cotransfected with pCIneo-huHSP27 and pCMV5-hGMEB1, or transfected with pCMV5-hGMEB1 alone. hGMEB1 was co-immunoprecipitated with HSP27 by anti-human HSP27 in cells expressing both proteins but not in cells expressing only hGMEB1 (Fig. 5A). Moreover, endogenous HSP27 present in extracts of CHO and HeLa cells bound to immobilized GST-hGMEB1 but not to GST-hGMEB1/CT or GST alone. Immobilized GST-hGMEB1, but not immobilized GST-hGMEB1/CT or GST alone, also pulled down myc-tagged hGMEB1 in extracts of pCIneo-myc-hGMEB1-transfected HeLa cells (Fig. 5B).

4. Discussion

The expression of the TAT gene has been extensively studied as a paradigm to understand the complex tissue-specific regulation of gene expression by multiple converging signaling pathways. Among others, two overlapping elements called TAT-GME and TAT-CRE of 21 and 26 bp, respectively, have been well characterized as important *cis*-acting enhancer modules [7,14]. TAT-CRE/GME contains an asymmetric CRE that was shown in vitro to bind CREB in a protein kinase A-mediated phosphorylation-dependent manner [8]. Together with a nearby hepatocyte nuclear factor-4 binding element, TAT-CRE/GME is sufficient in the liver environment to reproduce stimulation of TAT gene transcription by cAMP inducers such as glucagon [2]. TAT-CRE/GME is also known to increase the transcriptional activity mediated by the glucocorticoid receptor [12,14]. This activity is independent of CREB binding and instead depends on GMEB1 and GMEB2, two proteins that bind TAT-GME/CRE as a heteromeric complex of some 550 kDa [19]. A major role attributed to GME is to modulate glucocorticoid induction of target genes as a function of cell type and density. This characteristic of glucocorticoid-regulated genes, which has been characterized in detail for TAT, was reproduced with a minimal gene containing only a GME, a GRE and a minimal promoter, and shown to be dependent on the presence of GME [12,14–17]. Rat GMEB2 has been cloned recently [20], however, only partial tryptic peptide sequences have been obtained for rat GMEB1 [19]. In this paper, we reported the cloning and the characterization of a new protein binding to GME. The protein was designated hGMEB1 because it is the first reported human protein with a GME binding activity not competed by authentic CRE, it has the same apparent molecular weight and possesses identical sequences to tryptic fragments of rat GMEB1 [19], and it is closely related in sequence to rat GMEB2 [20].

hGMEB1 and GMEB2 possess little homology with other known nuclear proteins and probably belong to a new protein family of DNA binding proteins. The only significant homology with other proteins in the databases was found in an 80–90-amino acid domain described previously as the KDWK domain [28]. In this domain, hGMEB1 is 80% identical to rat GMEB2 and ca. 30% identical to a number of other nu-

clear proteins from human, rat, *Drosophila* and *Caenorhabditis elegans* (Fig. 2). The role played by the KDWK domain is unknown. The best characterized of the KDWK domain-containing proteins are *Drosophila* DEAF-1 and its likely mammalian orthologue, NUDR. DEAF-1 is a DNA binding protein that is thought to be a cofactor contributing to the autoregulation of the *Drosophila Deformed* gene [28]. NUDR has been isolated for its capacity to bind to a synthetic retinoic acid response element and is thought to be a cofactor that regulates transcription during the differentiation of testicular cells [29]. Other proteins with a homologous KDWK domain include mammalian Suppressin [30], a protein highly identical to NUDR, the nuclear body proteins SP140, LYSP100 and SP100 [31–33], two interferon-induced nuclear phosphoproteins of unknown function [34], and several uncharacterized *C. elegans* gene products [35]. hGMEB1 lacks a zinc finger domain and a leucine zipper motif and has no LXXLL motifs which mediate interactions of nuclear receptor coactivators with receptors [36]. Of possible significance is the existence of two glutamine-rich (24%) regions located in the central portion of hGMEB1. Glutamine-rich regions are found in several nuclear proteins and coactivators, and are likely to be involved in protein-protein interactions regulating transcriptional activation. For example, short glutamine-rich regions mediate the interaction of CREB with components of the TFIID complex [37,38]. The glutamine-rich regions of $TAF_{II}130$ are also required for binding to Sp1A, Sp1B and CREB, and for Sp1-mediated transcriptional enhancement [39]. hGMEB1 also possesses two serine/threonine-rich domains and also two putative α-helix coiled-coil domains, one of which extends over a glutamine-rich region (see Fig. 1B).

In rat, GMEB1 and GMEB2 have been shown to interact with each other and to bind the GME as a heteromultimeric complex of some 550 kDa. We have shown that this complex, which is shifted by the anti-hGMB1 antibody in the rat liver extract, is of the same size as the complex formed with in vitro translated hGMEB1, arguing that hGMEB1 can also multimerize with itself. This is further supported by the finding that hGMEB1 can be pulled down by immobilized GST-hGMEB1 (Fig. 5) and interacts with itself in the two-hybrid system (data not shown).

The significance of the interaction of HSP27 with hGMEB1 remains to be demonstrated. There is a well-documented role of heat shock proteins in the cell response to steroids. The glucocorticoid receptor is maintained in a high-affinity ligand binding conformation by associating with a heterocomplex formed of HSP90, HSP70 and other proteins. This association is essential to maintain a ligand binding conformation and may also be involved in the translocation of the activated receptor to the nucleus (for review see [4]). HSP27 belongs to the small heat shock protein family, a group of phylogenetically conserved proteins that includes αA- and αB-crystallin [40]. Intriguingly, proteins of this family are also under steroid regulation. Human HSP27 has been described in several reports as a p24 estrogen-induced protein and is overexpressed in several estrogen receptor-positive tumors [41–43]. αB-crystallin accumulates in NIH 3T3 cells in response to dexamethasone [44], and *Drosophila* HSP27 is induced by the steroid hormone ecdysone [45]. Several characteristics make HSP27 a good candidate as a modulator of the cell type- and cell density-modulatory properties reported for GME. HSP27 is expressed at highly variable levels in different cell lines [40] and it shows variation in its intracellular localization as a function of cell confluence [46]. Furthermore, HSP27 was first cloned in mouse as a cell growth-regulated protein [47]. A number of genes have been shown to undergo a left shift in dose-response curves induced by glucocorticoids or to be induced differentially as a function of confluence [16]. Our finding that hGMEB1, like HSP27, is a ubiquitously expressed protein suggests that its function is not restricted to the TAT gene or to the liver.

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