Cloning of a mouse glucocorticoid modulatory element binding protein, a new member of the KDWK family

Ana M. Jimenez-Lara, Mattias J.S. Heine, Hinrich Gronemeyer*

Institut de Génétique et de Biologie Moléculaire et Cellulaire, CNRS/INSERM/ULP, P.O. Box 163, 67404 Illkirch Cedex, France

Received 16 December 1999; received in revised form 28 January 2000

Edited by Jacques Hanoune

Abstract A mouse cDNA that encodes a nuclear DNA binding protein was identified by yeast two-hybrid screening using the activation domain 2 of the nuclear receptor coactivator TIF2 as a bait. BLAST analysis revealed that the identified cDNA encodes a KDWK domain and contains sequences almost identical to three tryptic peptides of rat GMEB-1 which together with the GMEB-2 heterodimeric partner binds to the GME/CRE sequence (glucocorticoid modulatory element) of the tyrosine aminotransferase (TAT) promoter. Mouse GMEB-1 is ubiquitously expressed in all the tissues examined. In vitro translated mGMEB-1 bound specifically to GME oligonucleotides, either alone or as a heterodimer with rGMEB-2. Transient transfection experiments with TAT promoter reporter genes suggest a potential role for mGMEB-1 as a transcriptional regulator of the TAT promoter.

Key words: TIF2; Glucocorticoid modulatory element binding protein; Transcriptional modulator; KDWK domain

1. Introduction

Nuclear receptors (NRs) are ligand-regulated transcription factors that comprise the receptors for steroid and thyroid hormones, retinoids and vitamin D [1–4]. Hormone binding induces a transconformation in the NR ligand binding domain involving the C-terminal helix H12 that encompasses the core of the conserved activation function 2 (AF2), thereby generating the surface for interaction with transcriptional coactivators [3–10]. Concomitantly, the interface between corepressors and the hormone free receptor is destabilized. Coactivators and corepressors recruit multiprotein complexes which, through modification of the acetylation status of nucleosomal histones, decondense or compact chromatin at target gene promoters [9–18]. While receptor-mediated chromatin decondensation appears to be required, but may not be sufficient for transcription activation [19], the ligand-dependent recruitment of another complex, TRAP/DRIP/SMCC, may mediate the effect of NR activation domains on the transcriptional machinery (for a review, see [20]). Bona fide NR coactivators such as SRC-1, GRIP1/TIF2, and pCIP/RAC3/ACTR/AIB1/TRAM1 contain two different transcription activation domains, AD1 and AD2 (for recent reviews, see [9,10]). AD1 acts through recruitment of the CREB binding protein (CBP) or the CBP-related protein p300. In addition to AD1, we and others have demonstrated the existence of a CBP-independent activation domain (AD2) located in the COOH-terminal region of, for example, TIF2 and ACTR [14,21].

Glucocorticoid receptors are ligand-dependent transcription factors that influence the transcriptional activity of cognate target gene programs, thus regulating diverse biological processes. The expression of the tyrosine aminotransferase (TAT) gene is triggered by glucocorticoids and glucagon, the latter acting via cAMP. The DNA enhancer element (glucocorticoid response element; GRE) through which the glucocorticoid receptor stimulates TAT gene transcription was initially identified at −2.5 kb upstream of the transcription start site [22,23]. A 26 bp cAMP response element (CRE) and a hepatocyte nuclear factor-4 binding site, separated by 50 bp at −3.6 kb, determine liver-specific and cAMP-dependent TAT gene activation; this is also the site of inhibition of TAT induction by insulin and phorbol esters via protein kinase C [24–31]. Recently, a further activity has been ascribed to the region harboring the TAT-CRE. In chimeric promoter reporter assays a 21 bp oligonucleotide encompassing the CRE was reported to cause a cell type- and density-dependent left shift in the glucocorticoid dose–response curve at sub-saturating concentrations. In the presence of this region, referred to as the glucocorticoid modulatory element (GME), also a positive transcriptional response to saturating concentrations of anti-glucocorticoids (e.g. dexamethasone-21-mesylate) was observed [32–37]. In a tandem arrangement together with a GRE in the background of a minimal promoter, the presence of the GME did not increase the maximum response to glucocorticoid but altered the EC_{50} values of the corresponding dose–response curve [33,38]. In vitro binding assays of protein–DNA interactions at the TAT-CRE/GME revealed two specific complexes which generated identical methylation interference patterns. It appears that the proteins involved in the formation of both complexes contact the same bases and bind with comparable affinities to the TAT-CRE/GME. One of these complexes has been shown to contain the cAMP response binding protein (CREB) [27,28]. The other corresponds to a multimeric complex composed of two proteins of 88 and 67 kDa denoted GME binding protein 1 (GMEB-1) and GMEB-2, respectively [38]. Rat GMEB-2 (rGMEB-2) belongs to a new family of nuclear factors that contain a highly conserved KDWK motif. The rGMEB-1 has not been cloned, but the amino acid sequence of three tryptic fragments revealed that rGMEB-1 seems to be a new protein unrelated to CREB [39].

In the present study, we report the isolation of a mouse cDNA encoding a novel KDWK motif-containing protein, highly homologous to rat GMEB-2 and with sequences almost identical to the three previously described tryptic pep-

*Corresponding author. Fax: (33)-3-88 65 32 01 or 32 76.
E-mail: hg@titus.u-strasbg.fr

PII: S0014-5793(00)01209-6

tides of rat GMEB-1. We present evidence that mGMEB-1 is an ubiquitously expressed nuclear protein. In vitro translated mGMEB-1 interacts specifically with the TAT-CRE/GME sequence, either alone or as heterodimer with rGMEB-2. Additionally, mGMEB-1 represses glucocorticoid-induced transcriptional activity from the TAT promoter depending specifically on the presence of the TAT-CRE/GME element. Our data suggest that GMEB-1 may correspond to a novel DNA binding transcriptional regulator belonging to a new family of *trans*-acting factors.

2. Materials and methods

2.1. Two-hybrid screening

The TIF2.2 deletion mutant (amino acids 1288–1464 of TIF2; encoding the activation domain AD2) was cloned in phase 3′ of the DNA binding domain of LexA in pBTM116 to produce a bait fusion protein. A mouse embryo (9.5–12.5 dpc) cDNA library in the λpASV3 phage yeast vector was used for screening [40]; amplification and plasmid excision (cre-*lox* system) was done in *Escherichia coli* BNN132. After plasmid excision cDNAs are expressed as VP16 acidic activation domain fusions. Two-hybrid screening [41] was done by sequential transformation of bait and library vectors in *Saccharomyces cerevisiae* L40a carrying two genomically integrated reporters, lexA-HIS3 and lexA-LacZ. After transformation, yeasts were plated on histidine-lacking selective medium containing 30 mM 3-amino-1,2,4-triazole (ATZ) and incubated at 30°C until transformants appeared. Transformants that grew more rapidly were restreaked in duplicate in selective medium and tested for β-galactosidase expression. One clone (C341) was selected for further analysis. Comparison of the C341 sequence with different databases strongly suggested that C341 is the mouse homologue of a previously described DNA-binding activity named GMEB-1. The full-length cDNA sequence of C341 clone was identified using a 5′ RACE system (Clontech).

2.2. Plasmids

The full-length cDNA sequence of C341/mGMEB-1 was subcloned into pSG5 [42] to obtain high levels of expression from the SV40 early promoter. GAL-GMEB-1 and VP16-GMEB-1 were constructed by cloning a *Bam*HI fragment containing full-length GMEB-1 cDNA into the *Bam*HI site of pG4M poly II and pSG5 NVP16 LB3 vectors, respectively. PG4M poly II contains GAL DBD preceding a polylinker with cloning sites for the cDNA, and stop codons in all reading frames. pSG5 NVP16 LB3 contains a cassette expressing a nuclear localized VP16 acidic activation domain (AAD) in the context of the pSG5 vector. rGMEB-2 was amplified by PCR from pcDNA 3.1 (ΔHisA)/rGMEB-2 [39] and inserted at the *Bam*HI site of the above described vectors to generate GAL-GMEB-2 and VP16-GMEB-2. *Bam*HI fragments of mGMEB-1 and rGMEB-2 were also subcloned into a HA-pSG5puro vector (gift from A. Benecke) to produce a tagged protein with the hemagglutinin epitope at the amino-terminus. HA-pSG5puro is a puromycin-modified pSG5 vector (constructed by C. Erb) that contains a hemagglutinin epitope N-terminal to a multicloning site. B-GMEB-1 and B-GMEB-2 were constructed by cloning a *Bam*HI fragment into the pSG5puro-BNtag vector (gift from T. Lerouge). pSG5puro-BNtag directs synthesis of epitope (region B of human estrogen receptor (ER))-tagged fusion proteins. Details concerning the plasmid constructions, all of which were verified by sequencing, are available on request.

2.3. Northern blot analysis

A 437 bp PCR fragment encompassing nucleotides 117–557 of C341 was ^{32}P-labeled using the Klenow fragment of polymerase. This probe was hybridized to a mouse mRNA blot (MTN; Clontech) using the ExpressHyb solution (Clontech) provided by the manufacturer.

2.4. Transient transfection

Transient transfections were performed using the calcium phosphate co-precipitation protocol in the presence of media with charcoal-treated serum. Cells were incubated with plasmid DNA for 14–16 h before being induced with dexamethasone (Sigma) for 24 h. Transfected cells were lysed and assayed for reporter gene activity using the CAT immunodetection system (Boehringer Mannheim) according to the manufacturer's instruction. β-Galactosidase, measured as previously described [21], was used to normalize CAT measurements.

2.5. Immunofluorescence microscopy of mGMEB-1

Immunofluorescence was performed in Cos-1 cells growing on glass coverslips. Cells overexpressing HA-pSG5puro-mGMEB-1 or the parental control vector were fixed in 4% formaldehyde for 10 min and permeabilized with PBS/0.2% Triton X-100/5% BSA for 30 min at room temperature. The presence of GMEB-1 was revealed through its HA epitope using the monoclonal anti-HA antibody 12ca5 diluted 1/1000 in PBS/0.2% Triton X-100/1% BSA; the antigen–antibody complexes were revealed with Texas red-labeled anti-mouse IgG (CY3) diluted 1/300. Nuclei were stained with 5 μg/ml Hoechst 33258.

2.6. Electrophoretic mobility shift assay

In vitro transcription/translation to produce recombinant proteins was done with the TNT Coupled Reticulocyte Lysate System (Promega, Madison, WI, USA) using 1 μg of the plasmids HA-GMEB-1, HA-GMEB-2, B-GMEB-1 and B-GMEB-2, according to the supplied instructions.

Recombinant proteins were incubated on ice with poly(dI-dC) (50 ng) as non-specific competitor in a 20 μl reaction containing 50 mM KCl, 20 mM HEPES (pH 7.3), 2 mM DTT, 5% glycerol, and 0.1% Triton X-100. After overnight incubation at 4°C, ^{32}P-labeled probe was added and incubated for an additional 20 min at 25°C. Protein–DNA complexes were separated from free probe on a 4% non-denaturing polyacrylamide gel by electrophoresis at 250 V for 2 h. The dried gels were autoradiographed for 16–24 h at −80°C using Kodak X-omat films. In supershift analyses, the antibody against the hemagglutinin epitope (12ca5) or against the epitope of the estrogen receptor B region (B10) was added to the recombinant proteins 15 min on ice before addition of the radiolabeled probe. The GME probe used in this study (5′-CTTCTGCGTCAGCGCCAGTAT-3′) corresponds to the rat TAT promoter spanning position −3654 to −3634. GMEmut is an mutant probe (5′-CTTCTG**TATG**AGCGCCAGTAT-3′) previously described [36].

3. Results

3.1. Cloning and sequence analysis of mouse GMEB-1, a member of the KDWK family

The yeast two-hybrid system [41] was used to isolate cDNAs encoding mouse proteins that interact specifically with the C-terminal region of TIF2. The activation domain AD2 of TIF2 encompassing amino acids 1287–1464 was fused to the DBD of LexA. The resulting hybrid protein (LexA-TIF2.2; Fig. 1A) was expressed in the yeast reporter strain L40a which harbors HIS3 and LacZ reporter genes, both under the control of *E. coli* LexA promoters, thus allowing a double selection strategy by using auxotrophy selection in medium lacking histidine and identification of positive clones by β-galactosidase activity. A randomly primed mouse embryo cDNA library was constructed in the yeast multicopy expression vector pΔSV3 [40], such that the polypeptides are expressed as fusion proteins with the AAD of the VP16 protein, and subsequently introduced into L40a expressing LexA-TIF2.2. Resulting yeast transformants containing both plasmids were plated on synthetic minimal medium lacking histidine but containing 30 mM of ATZ. The latter compound represses residual constitutive bait activity to overcome the problem of false positives. Quickest growing positive transformants were identified and tested for β-galactosidase activity. The corresponding mouse cDNAs were re-tested to confirm TIF2 AD2 interaction using a second two-hybrid system based on ER(DBD) chimeras and the ERE-URA3 reporter [43]. Among the clones identified, C341 was selected for further characterization. As shown in Fig. 1, the presence of

clone C341 was sufficient to allow yeast cells to grow on medium lacking histidine (Fig. 1B). Activation of the LacZ reporter gene was determined by measuring β-galactosidase activity (Fig. 1C). When expressed alone neither LexA-TIF2.2 nor VP16-C341 transactivated the LacZ reporter above levels seen with the DBD or the AAD alone. In contrast, coexpression of the two hybrid proteins resulted in a strong expression of the reporter gene, indicating that clone C341 and the AD2 activation domain of TIF2 interact in yeast. Thus, we concluded that C341 corresponds to a protein that, through direct or indirect interactions with TIF2 AD2, increased the transcriptional activity of the LexA-TIF2.2 fusion protein on its cognate reporter gene, suggesting that C341 acts as co-activator of TIF2 AD2 in this particular system.

The originally isolated C341 revealed a 2.4 kb sequence encoding a 540 amino acid peptide in frame with the acidic activation domain of VP16. A stop codon and a poly-A signal in the 3′ untranslated sequence suggested that the 3′ end of

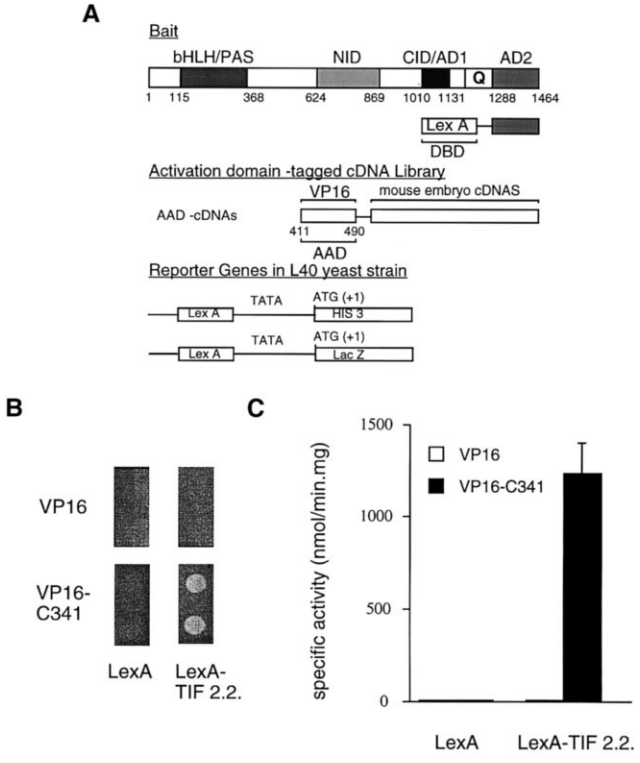

Fig. 1. Isolation of GMEB-1 by two-hybrid assay. A: A schematic representation of functional domains of TIF2 as well as the Lex chimera used as a bait in the yeast two-hybrid screen. bHLH, basic helix-loop-helix sequence; PAS, Per–Arnt–Sim domain; CID, CBP interacting domain; AD, activation domain. The VP16 AAD-tagged mouse embryo cDNA expression library is represented below. Transcription of the integrated HIS3- and LacZ-based reporter genes is regulated by a LexA promoter in the yeast reporter strain L40a. B: Growth of transformants coexpressing LexA-TIF2.2 chimera and clone C341 on selective medium. Negative controls (top panel and bottom left panel) are also shown. The growth phenotypes of the transformants were assayed using a spot test on medium lacking histidine but containing 30 mM ATZ. Note that expression of the VP16-C341 chimera, but not of VP16, allows yeast cells to grow on selective medium provided LexA-TIF2.2 is expressed. Plates were incubated for 2 days and photographed. C: β-Galactosidase levels in L40a yeast strain expressing LexA (DBD) or the LexA-TIF2.2 fusion protein in the presence of VP16 or VP16-C341. The LacZ reporter gene is under the control of a LexA promoter.

```
ATGCTTCTCGTTCCCCGCAGCAGCCCCAGCTAGCTGACTTCATGTGAAAGATGGCCAATG
                                                        M  A  N  A
CAGAAGTGAGCGTCCCAGTGGGAGATGTGGTTGTGGTACCCACTGAAGGAAATGAAGGGG
  E  V  S  V  P  V  G  D  V  V  V  V  P  T  E  G  N  E  G  E
AGAACCCGGAAGACACTAAAACTCAAGTGATCTTGCAGTTACAGCCTGTGCAACAAGGGA
  N  P  E  D  T  K  T  Q  V  I  L  Q  L  Q  P  V  Q  Q  G  I
TTTATGAAGCTGGGTCGGAGAATAGCGCAGCAGTTGTAGCCGTAGAGACCCACTCAATAC
  Y  E  A  G  S  E  N  S  A  A  V  V  A  V  E  T  H  S  I  H
ACAAAATCGAGGAAGGAATTGATGCAAGCAGTATAGAAGGGAACGAGGACATGGAGATCG
  K  I  E  E  G  I  D  A  S  S  I  E  G  N  E  D  M  E  I  A
CTTACCCTATAACCTGTGGGGAGAGTAAAGCTGTCCTCCTCTGGAAGAAGTTCGTGTGTC
  Y  P  I  T  C  G  E  S  K  A  V  L  L  W  K  K  F  V  C  P
CAGGAATAAACGTGAAGTGTGTCAAGTTTAATGACCAGCTGATCAGTCCCAAGCACTTCG
  G  I  N  V  K  C  V  K  F  N  D  Q  L  I  S  P  K  H  F  V
TTCACCTGGCTGGCAAGTCCACCCTGAAGGACTGGAAGAGAGCCATCCGTCTAGGCGGCA
  H  L  A  G  K  S  T  L  K  D  W  K  R  A  I  R  L  G  G  I
TCATGCTCAGGAAGATGATGGACTCTGGACAGATCGATTTTTACCAGCATGACAAAGTTT
  M  L  R  K  M  M  D  S  G  Q  I  D  F  Y  Q  H  D  K  V  C
GCTCCAATACCTGCAGGAGCACCAAATTTGACCTCCTGATCAGCAGTGCGAGGGCTCCAG
  S  N  T  C  R  S  T  K  F  D  L  L  I  S  S  A  R  A  P  V
TGCCAGGACAGCAGACAAGTGTGGTGCAGACCCCCACCTCGGCCGATGGGAACATCACAC
  P  G  Q  Q  T  S  V  V  Q  T  P  T  S  A  D  G  N  I  T  Q
AGATCGCCATCTCAGAGGAGAGCATGGAAGAGGCAGGGCTGGAGTGGAACTCAGCCCTCA
  I  A  I  S  E  E  S  M  E  E  A  G  L  E  W  N  S  A  L  T
CCGCCGCTGTCACCATGGCCACAGAGGAGGGTATAAAGAAAGAGTCTGAAGAGATTTCAG
  A  A  V  T  M  A  T  E  E  G  I  K  K  E  S  E  E  I  S  E
AGGACACTTTGATGTTCTGGAAGGGGATAGCTGATGTAGGGTTGATGGAGGAGGTCATCT
  D  T  L  M  F  W  K  G  I  A  D  V  G  L  M  E  E  V  I  C
GCAACATCCAGAAGGAGATGGAAGAGCTGCTCAGGGGCGTTCAGCAGCGGCTCATTCAGG
  N  I  Q  K  E  M  E  E  L  L  R  G  V  Q  Q  R  L  I  Q  A
CCCCTTTCCAGGTCACAGATGCTGCTGTTCTAAACAATGTGGCGAACACATTTGGCCTAA
  P  F  Q  V  T  D  A  A  V  L  N  N  V  A  N  T  F  G  L  M
TGGACGCGGTCAAGAGAGTTTTGGACAACAGACGGAAGCAAGTGGAGCAGGGCGAGGAGC
  D  A  V  K  R  V  L  D  N  R  R  K  Q  V  E  Q  G  E  E  Q
AGTTTCTCTACACCCTGGCAGACTTGGAACGGCAGTTGGAGGAGCAAAAAAAGCAAGCCC
  F  L  Y  T  L  A  D  L  E  R  Q  L  E  E  Q  K  K  Q  A  Q
AAGATCCCAGACTGAAATCCCAGACAGTTCAAAATGTGGTACTGATGCCCGTGAGCACCC
  D  P  R  L  K  S  Q  T  V  Q  N  V  V  L  M  P  V  S  T  P
CAAAGCCTCCAAAAAGACCCCGGCTCCAGCGGCCAGCCTCCACCACCGTCCTGAGCCCTT
  K  P  P  K  R  P  R  L  Q  R  P  A  S  T  T  V  L  S  P  S
CTCCTGTCCAGCAGCCTCAGTTTACTGTCATCTCTCCCATCACCATTACTCCTGTGGGCC
  P  V  Q  Q  P  Q  F  T  V  I  S  P  I  T  I  T  P  V  G  Q
AGTCCTTCTCCATGGGCAACATCCCAGTGGCTACCCTCAGCCAGGGCTCCAGTCCTGTGA
  S  F  S  M  G  N  I  P  V  A  T  L  S  Q  G  S  S  P  V  T
CTGTCCACACACTGCCTTCTGGCCCTCAGCTCTTCCGCTATGCCACGGTGGTTTCCTCTG
  V  H  T  L  P  S  G  P  Q  L  F  R  Y  A  T  V  V  S  S  A
CCAAGAGCAACTCACCAGACACAGTGACCATCCACCCTTCCTCAAGTTTGGCACTGCTAA
  K  S  N  S  P  D  T  V  T  I  H  P  S  S  S  L  A  L  L  S
GCTCCACCTCTATGCAGGATGGGAGTTCCCTGGGCAACATGGCCACCATGGTGAGCCCCA
  S  T  S  M  Q  D  G  S  S  L  G  N  M  A  T  M  V  S  P  M
TGGAGCTGGTGGCCATGGAGTCCGGCCTGACGTCAGCAATCCAGGCTGTTGAAAGCACCT
  E  L  V  A  M  E  S  G  L  T  S  A  I  Q  A  V  E  S  T  S
CTGAGGATGGGCAGACCATCATTGAAATCGACCCTGCCCCAGACTCCGAGGCTGACGACA
  E  D  G  Q  T  I  I  E  I  D  P  A  P  D  S  E  A  D  D  T
CTGAGGGCAAAGCAGTCATCTTGGAGACAGGGCTGAGGACTGAGGAGAAAGTGGTAGCCG
  E  G  K  A  V  I  L  E  T  G  L  R  T  E  E  K  V  V  A  E
AAATGGAAGAACACCAGCATCAGGTCCACAATGTGGAGATTGTGGTCTTGGAGGACTGAT
  M  E  E  H  Q  H  Q  V  H  N  V  E  I  V  V  L  E  D  *
TGGGGACAGGGCCAAGAGCTGTGTTTTGGTTTGGAATTTTAATTATGTTTATTTTTATCA
TTGTCCCGCTCATTTCCACATAGAACTTTTTTTAAAAAAAAAAACAAAAACAAACAAAAA
AAAACCAAACTACAAAATCTCGTTGTAACTGCAAATGTTGGGTTCCTCCTACTTCCTCAT
TGACAACTGGACAGAACAAGCTGCCTTTCCAGAAGTTAGAAACAGGGCACCCAGGGTCTG
AACCATCTTTCCCCAAGGAAGGTAATTGCTTTTAGGGGAAGTGTCAAAATATCCAGGAAT
CCTTTCCAGAATAGTCTGCCTGTGTATACACGCATGGTCTCACTCTTGTAAAGACGCATT
GACCAGACCCTGGAACACAGATCTGACTCCAGAAGATGACCAGCTCTGCTGTGGATACAG
AAGCACGCTTGCCTCTGTGTCCTGGATATGATGGCCTCAGATGCTGGTGTGCAAAACTGA
AAGAACAGGAGGTTGGCTCTGCTTAGGGTGGAAAGTAGTGTGATCAGCAGCTCATAGGGG
GAGCCCTTTGACAGGGAGGGGCCGAGGAATGGTCTTCCAGAGGTGCATCTGATGCCCCTT
GCGCTCTCTGAGGGTTGGGTACCATTTTCAATTTCAGAAGAGGACAGAGATTATAGAAAG
ACACACAGCAATCAAAAGATTGTCACAGCTGTGCTTTTGGGATAGCTCACCTTGCCCCCA
GCCTTGTCAAAGCCCTCACCCTCCTC
```

Fig. 2. Nucleotide and deduced amino acid sequences of mouse GMEB-1 (GenBank accession number AF210433). Box refers to the homologous region between GMEB-1 and GMEB-2. Conserved KDWK motif is shown in bold. Underlined sequences identify three partially sequenced tryptic fragments described by Oshima et al. [38].

the cDNA had been cloned, whereas the start codon was lacking. A 5′ RACE led to the identification of additional 5′ sequences encompassing the start codon and 50 bp of 5′ UTR. The complete nucleotide and amino acid sequences of mGMEB-1 cDNA are presented in Fig. 2. The cDNA sequence predicts an open reading frame of 1689 bp corresponding to a polypeptide of 562 amino acids.

A BLAST search [44] of the SwissProt and EMBL databases revealed a very closely related sequence termed glucocorticoid modulatory element protein, GMEB-2. The search also identified a sequence with weak homology to a number of other peptide sequences with unknown function. Multiple sequence alignment of the homology regions (CLUSTALW

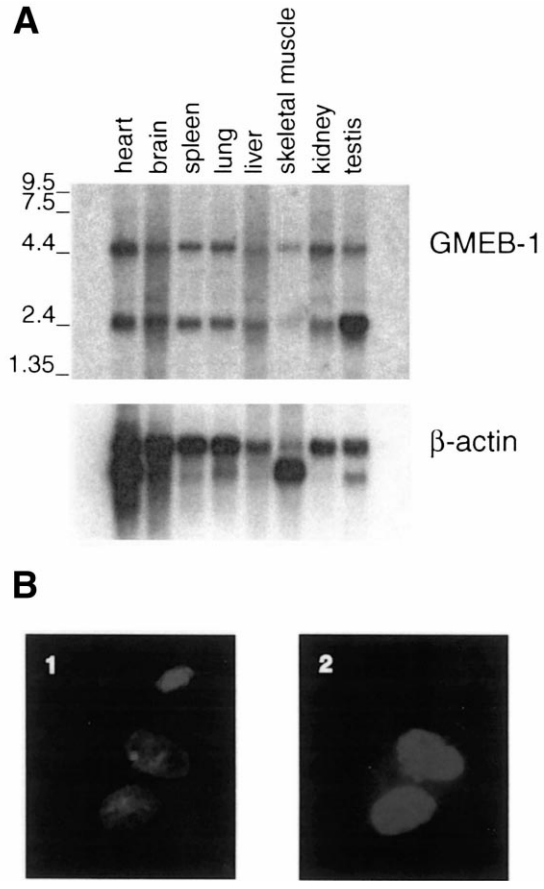

Fig. 3. A: Expression of GMEB-1 in various mouse tissues. Mouse Multiple Tissue Northern Blot (Clontech) was probed with a mGMEB-1 cDNA fragment probe harboring the KDWK motif. The blot was stripped of the probe after autoradiography and re-probed with a β-actin cDNA probe. The position of GMEB-1 and β-actin is indicated on the right. Numbers on the left indicate the position of molecular weight markers. B: Cellular localization of GMEB-1. Panel 2 shows Cos-1 cells transfected with HA-pSG5-GMEB-1, fixed and processed for immunofluorescence microscopy using anti-hemagglutinin antibody. Panel 1 shows a Hoechst staining of the same field.

program) revealed the presence in C341 of a conserved motif, previously described by Gross and McGinnis as 'KDWK motif' [45,46].

Rat GMEB-2 and C341 exhibit 61% sequence identity in the region (aa 78–217) harboring the KDWK motif. rGMEB-2 has been reported to bind to the TAT-CRE/GME as a multimeric complex together with the 88 kDa rat GMEB-1 protein, which has been previously purified to homogeneity. Two of three tryptic peptides of rGMEB-1 [38] are identical to residues 123–129 and 183–195 of C341 (Fig. 2); 17 of 18 residues of the third peptide are conserved. Taken together, the sequence similarity with rGMEB-2 and the identity with the three tryptic fragments of rGMEB-1 suggest that the C341 protein corresponds to the mouse homologue of GMEB-1.

3.2. GMEB-1 is a ubiquitously expressed nuclear protein

Northern blot analysis of NIH3T3 cells using as probe a sequence located in the 3′ UTR of C341 showed that the predominant GMEB-1 mRNA has a size of approximately 5 kb. A second band, with an estimated size of 2.4 kb, was also observed when a sequence that contains the KDWK motif was used as probe (data not shown). Examination of various mouse tissues for GMEB-1 expression revealed that both the 5 kb and the 2.4 kb mRNAs are expressed in all tissues tested (Fig. 3A). In testis the 2.4 kb mRNA is the most abundant transcript. Whether this shorter GMEB-1 RNA represents an alternative splice variant of a single GMEB-1 gene or a RNA transcript from a related protein, e.g. GMEB-2, is under investigation.

The intracellular localization of GMEB-1 was determined by immunofluorescence in Cos-1 cells transfected with a vector expressing an HA-tagged GMEB-1. Fig. 3B shows that HA-tagged GMEB-1 is an exclusively nuclear protein.

3.3. GMEB-1 binds to regulatory elements within the tyrosine aminotransferase promoter

Since GMEB-1 was initially identified as a member of a multimeric complex that binds to the GME/CRE sequence present in the promoter region of the tyrosine aminotransferase gene [38], we tested whether mGMEB-1 could directly interact with the GME/CRE motif. In vitro produced HA-

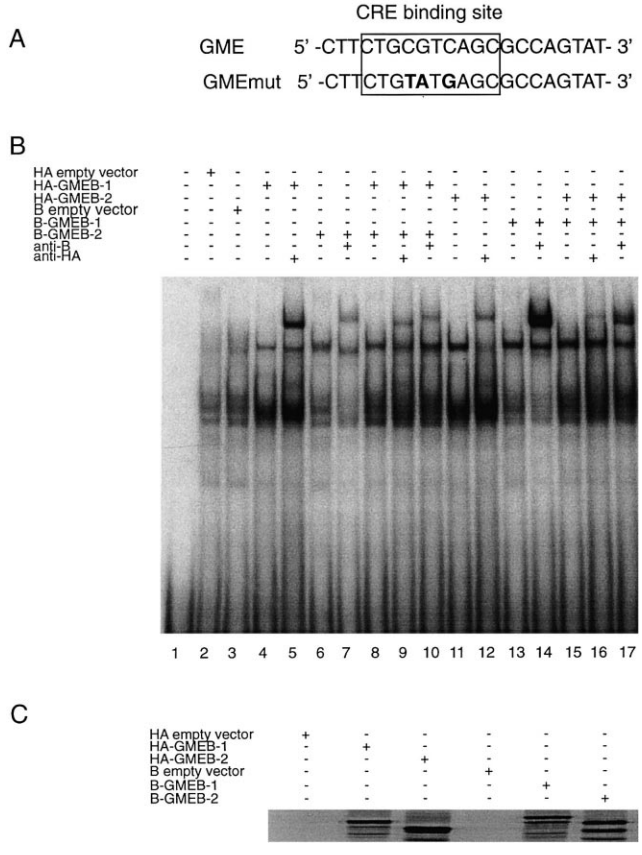

Fig. 4. A: Sequence of GME oligonucleotides used for gel retardation experiments. Mutated residues which impair CRE and GMEB binding are indicated in boldface. The boxed sequence corresponds to the CRE binding site in the tyrosine aminotransferase promoter. B: Binding of GMEB-1 to GME oligonucleotide in gel shift assays. Protein–DNA complexes were separated by non-denaturing electrophoresis after incubating a ^{32}P-labeled GME oligonucleotides with unprogrammed reticulocyte lysates or programmed reticulocyte lysates (expressing HA-GMEB-1, HA-GMEB-2, B-GMEB-1, or B-GMEB-2), as indicated at the top of the figure. Supershift assays were performed with anti-hemagglutinin (anti-HA) or anti-B epitope antibodies. In C protein levels of [^{35}S]methionine-labeled tagged GMEB-1 and GMEB-2 used for the gel shift assays in B are shown to demonstrate that the two proteins are expressed at similar levels.

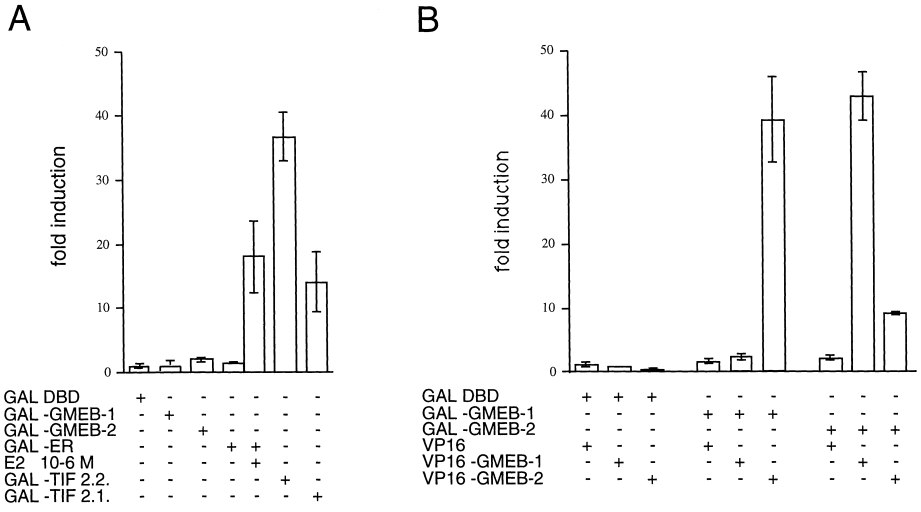

Fig. 5. Activity and interactions of GMEBs. Activity of GMEB chimeras in mammalian one- (A) and two-hybrid (B) assays. Cos-1 cells were co-transfected with 1 μg of the (17m)5-TATA CAT reporter gene and 0.2 μg of the GAL and VP16 chimeras as indicated. Each bar represents the mean value obtained from at least three different experiments, standard deviations are indicated.

or B-tagged GMEB-1 (termed 'HA-GMEB-1' and 'B-GMEB-1') as well as HA- and B-tagged GMEB-2 ('HA-GMEB-2' and 'B-GMEB-2') were tested in gel shift assays using ^{32}P-labeled GME (Fig. 4A). GMEB-1 produced a discrete band shift, which was further retarded in the presence of anti-hemagglutinin antibodies. That GMEB-1 bound the GME in a sequence-specific manner was obvious from experiments using GMEmut to which GMEB-1 was unable to bind. Note that GMEmut corresponds to a mutated GME sequence known to bind neither GMEBs nor CREB ([36] and data not shown).

The DNA binding activity of GMEB-1 was also tested in the presence of an in vitro translated N-terminally tagged GMEB-2 containing an epitope of the estrogen receptor B region. When GMEB-1 and GMEB-2 were mixed a GME complex was formed that migrated with a mobility distinct from that of the corresponding GMEB-1 or GMEB-2 complexes. Supershift analysis with anti-hemagglutinin and anti-B epitope confirmed the presence of both proteins in the DNA complex. However, no cooperative DNA binding was observed when both proteins were present. Similar results were obtained with HA-GMEB-2 and B-GMEB-1 (Fig. 4, lanes 11–17). Fig. 4B shows a SDS–PAGE of ^{35}S-labeled in vitro translated proteins to confirm that similar levels of proteins were used in those gel shift assays.

3.4. GMEB-1 interacts with GMEB2

To examine the intrinsic transactivation activity as well as the homo- and heterodimerization properties of GMEB-1, the full-length cDNAs of GMEB-1 and GMEB-2 were fused to either the GAL-4 DNA binding domain or to the VP16 activation domain. In Cos-1 cells none of the GAL-GMEB fusion proteins was able to transactivate a cognate reporter gene as no significant difference was seen between the GAL DBD and the GAL-GMEB-1 or GAL-GMEB-2 (Fig. 5A). In contrast, transactivators such as the ER AF2 in the presence of estradiol or the TIF2 ADs, which were used as positive controls linked to the GAL DBD, strongly activated transcription from the same promoter demonstrating the presence of transactivation functions in these proteins (Fig. 5A).

A mammalian two-hybrid assay was used to examine the

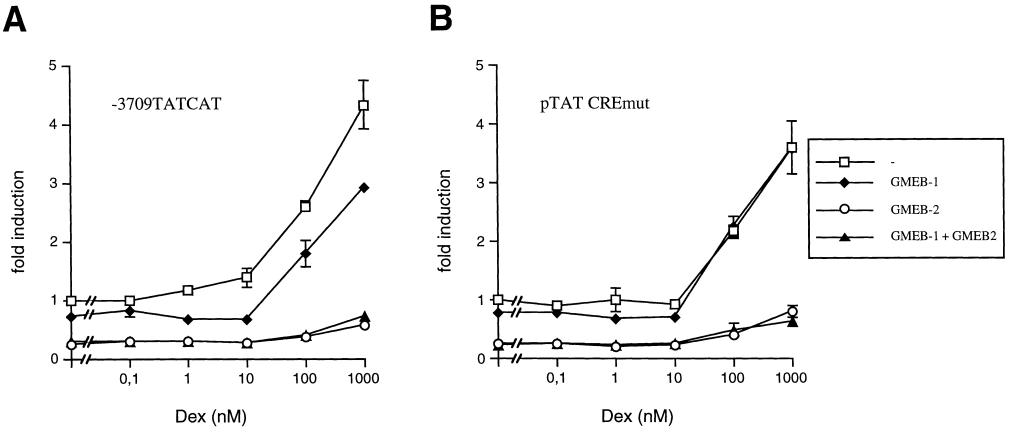

Fig. 6. Effect of GMEB-1 and GMEB-2 overexpression at increasing concentrations of dexamethasone (Dex). Reporter plasmids were transfected into HepG2 cells and induced with the indicated concentrations of Dex in the presence or absence of 1 μg expression vector for the GMEBs as indicated. After 24 h cells were harvested and the CAT activities in cell lysates were determined as described in Section 2. Each point represents the mean of the fold induction obtained from triplicate experiments.

possible in vivo interaction between GMEB-1 and GMEB-2. When GMEB-1 and GMEB-2 chimeras were cotransfected with a cognate GAL-4 reporter gene a highly increased transactivation was observed (Fig. 5B). These results indicate the ability of these proteins to heterodimerize and thus are in keeping with the above DNA binding results. Note that GMEB-2 also has a significant ability to homodimerize while GMEB-1 homodimerizes weakly, if at all.

3.5. GMEB-1 represses transcription from the tyrosine aminotransferase gene promoter

As the GME/CRE sequence of the TAT promoter has previously been described as a *cis*-acting element that causes a left shift in the dose–response curve of the TAT gene promoter to sub-saturating concentrations of glucocorticoids [32,34,35,37,38], we investigated the potential effect of GMEBs on the transactivation of chimeric TAT promoter reporter genes comprising the GME linked to the chloramphenicol acetyltransferase reporter gene [27]. As shown in Fig. 6A we analyzed the effect of GMEB-1 and GMEB-2 overexpression on the dose–response curve of the TAT promoter to dexamethasone. In all cases, overexpression of GMEBs produced a repression of both the basal and the dexamethasone-induced transcription of the TAT gene promoter; this repression was more pronounced in the presence of GMEB-2 than in the presence of GMEB-1.

To further examine the contribution of the GME/CRE sequence to GMEB-1-modulated transactivation, we studied the effect of the overexpression of GMEB-1 on a promoter reporter gene in which the GME/CRE sequence was mutated [27]. Interestingly, GMEB-1 did not affect TAT transcription at any concentration of dexamethasone tested. By contrast, a repressive effect was observed on the basal level of transcription as it was seen on the wild-type promoter. On the other hand, overexpression of GMEB-2 repressed TAT promoter transcription in a CRE/GME-independent manner at all the concentrations tested. Additionally, overexpression of both proteins had the same repressive effect as overexpression of GMEB-2 alone. These results suggest that GMEB-1 repression of dexamethasone-induced TAT activity appears to be dependent on DNA binding to the GME/CRE sequence.

4. Discussion

4.1. GMEB-1 acts as a TIF2 AD2 cofactor in yeast but not in mammalian cells

The two TIF2 activation functions, AD1 and AD2, apparently operate through different transcriptional activation cascades. While the TIF2 AD1 activation domain could not be separated by mutational analysis from the TIF2 domain which interacts in vitro and in vivo with a region of the CBP surface, neither this region nor the full-length CBP interacted with the TIF2 AD2 [21]. In an attempt to define the mechanism mediating TIF2 AD2 activity, we have identified a new mouse cDNA and have initiated the characterization of the encoded protein. Interestingly, a yeast two-hybrid analysis shows a strong functional interaction between the TIF2 AD2 activation domain and GMEB-1 in yeast when they were tested in a two-hybrid system. However, this interaction has not been observed using a similar approach in a mammalian system. Additionally, no physical interaction was found in vitro when these two proteins were tested in GST pull-down experiments (data not shown). Apparently, these observations suggest that yeast cells contains some factor(s) that establish a link between GMEB-1 and TIF2 AD2. It is possible that homologous factors exist in mammalian cells but are expressed in a cell-specific manner. However, we can also not exclude the possibility that specific post-transcriptional modifications are required for this interaction. Such modifications may occur in yeast due to the conservation of the appropriate enzymatic machinery, but may be only cell-specifically active in certain mammalian cells.

4.2. GMEB-1 belongs to an emerging family of polypeptides which share a KDWK domain

A sequence homology search with C341 revealed the highest homology with the rat GMEB-2 peptide sequence [39]. Moreover, clone C341 encodes three tryptic fragments of the rat GMEB-1, the dimerization partner of GMEB-2, thus strongly suggesting that clone C341 is mouse GMEB-1. GMEB-1 and GMEB-2 possess little homology with other known nuclear proteins. The only significant homology with other known proteins was found in an 80–90 amino acid domain described previously as the KDWK domain [46]. This domain appears in a number of nuclear proteins from different species, such as human, rat, *Drosophila* and *Caenorhabditis elegans*. Significantly, no matches were found in the genomes of prokaryotes or eukaryotic unicellular organisms, suggesting that this gene family is a feature of metazoans. The role played by the KDWK domain is unknown. The best characterized KDWK proteins are the *Drosophila* DEAF-1 protein [46] and NUDR (for 'nuclear DEAF-1 related') [47]. *Drosophila* DEAF-1 is a DNA binding protein that has been shown to be an important cofactor in Deformed (*Dfd*) gene expression during embryonic development. NUDR is a nuclear regulatory factor identified by affinity binding to a synthetic retinoic acid response element. The sequence and functional similarities between NUDR and DEAF-1 suggest that NUDR may also act as a cofactor to regulate the transcription of genes during fetal development or differentiation of testicular cells [47]. Others proteins with a homologous KDWK domain include mammalian Suppressin [48], a protein highly related to NUDR, the nuclear body proteins SP100, Lysp100 and SP140 [49,50], two interferon-induced nuclear phosphoproteins of unknown function [51], and several uncharacterized *C. elegans* gene products [52]. Significantly, most of these proteins have been reported to exhibit aberrant migration rates when analyzed by SDS–PAGE. The calculated molecular mass of GMEB-1 is 65 kDa but the protein produced by in vitro transcription/translation migrates with an apparent molecular mass of 85 kDa (data not shown). Differences between the electrophoretic mobility of proteins and their actual molecular mass can be attributed either to post-translational modifications of the translated products or to specific amino acid regions that may bind SDS in an anomalous fashion, probably the KDWK domain may be responsible for this anomaly in this protein family [53].

4.3. Does GMEB-1 function as a transcriptional regulator in the tyrosine aminotransferase promoter?

Zeng et al. [39] have shown that the rat homologues of GMEB-1 and GMEB-2 interact with each other and bind the GME sequence present in the tyrosine aminotransferase gene promoter as a heterodimeric complex. In line with this,

our experimental data support that GMEB-1 is a nuclear protein with some DNA binding properties. In vitro, GMEB-1 alone or as heterodimer with GMEB-2 binds to the GME sequence in a specific manner, as mutation of three residues in this element abolishes both GMEB-1 and GMEB-1/GMEB-2 binding. However, we did not find any structural homology with known DNA binding motifs (e.g. zinc fingers), despite some cysteine clustering in the N-terminal part of GMEB-1 [54,55]. Both GMEB polypeptides are acidic, with a particularly high concentration of acidic residues in their carboxy-terminal regions, which are also rich in proline, serine, and threonine, suggesting that these could be transactivation domains. However, we were unable to find conditions, if they exist, in which recruitment of GMEBs to a promoter would result in transcription activation of the corresponding gene and we were thus unable to detect the presence of an autonomous transcription activation domain in GMEBs. However, we cannot exclude that additional signalling pathways may be required to confer transcriptional activation potential on the GMEBs. In this context, we note the presence of putative phosphorylation sites for several serine/threonine kinases in these proteins. The fact that GMEB-1 shares certain properties with conventional transcriptional regulators (i.e. heterodimerization with related proteins and specific DNA binding) but does not show an intrinsic activation domain under conditions where other activators do, suggests that (i) GMEB-1 can act as a DNA binding factor without a real activation function, i.e. GMEB-1 could be a modulator of the DNA binding activity of other transcriptional factors or it could harbor an activity required for the modification of different proteins involved in transcription, or (ii) the GMEB-1 transactivation function acts in a cell-specific manner and we have not tested the proper cells. This latter hypothesis would be in keeping with the yeast results and would point to an evolutionarily conserved pathway for GMEB activation.

As mentioned before a major role attributed to GMEBs is to modulate glucocorticoid induction of the TAT gene as a function of cell type and density. In view of this general assumption we expected that overexpression of GMEBs could promote a left shift in the dose–response curve for dexamethasone induction of the tyrosine aminotransferase gene promoter, as described previously [37]. However, in our experimental setting GMEB overexpression repressed TAT promoter activity at all concentrations of dexamethasone tested. The same effect was seen for non-induced transcription. It is possible that overexpression of GMEBs can cause the sequestration of some limiting factors required for TAT gene transcription in HepG2 cells, resulting in squelching phenomena [56–59]. This possibility is supported by the observation that GMEB-2 inhibited TAT promoter activity even in pTAT-CREmut, suggesting a DNA binding-independent action of GMEB-2.

While the analysis of mGMEB-1 was carried out, Theriault et al. isolated the human homologue of GMEB-1 using the heat shock protein 27 (HSP27) as bait in a yeast two-hybrid assay [60]. In contrast to the present study, Theriault et al. could monitor direct HSP27-GMEB-1 interaction. Understanding how GMEB-1 can interact with two apparently distinct baits will require identification of the HSP27-GMEB-1 interaction surface and the definition of the interaction mode of TIF2 and GMEB-1. Additionally, cloning of GMEB-1 as a parvovirus initiation factor (PIF), essential for viral replication, was reported while this article was under review [61]. The DNA binding-based cloning of GMEB-1/PIF can be understood from the similarity of PIF and ATF/CRE binding sites (see [61] for details). However, it remains to be studied whether GMEB-1/PIF serves a dual function or whether this DNA binding factor is simply 'misused' by the viral replication machinery.

Acknowledgements: We are grateful to S. Stoney Simons Jr. for the generous gift of the rat GMEB-2 mammalian expression vector, A. Pornon and C. Erb for excellent technical assistance, and the sequencing and cell culture services for help. M.J.S.H. is currently with Boehringer Ingelheim, Germany. A.M.J.-L. and M.J.S.H. are recipients of Spanish Education Ministry and Boehringer-Ingelheim fellowships, respectively. This work was supported by funds from the Institut National de la Santé et de la Recherche Médicale, the Centre National de la Recherche Scientifique, the Hôpital Universitaire de Strasbourg, the EC BIOMED programme (Grant BMH4-96-0181 to H.G.), and Bristol-Myers Squibb.

References

[1] Mangelsdorf, D.J., Thummel, C., Beato, M., Herrlich, P., Schutz, G., Umesono, K., Blumberg, B., Kastner, P., Mark, M. and Chambon, P. (1995) Cell 83, 835–839.
[2] Beato, M., Herrlich, P. and Schutz, G. (1995) Cell 83, 851–857.
[3] Chambon, P. (1996) FASEB J. 10, 940–945.
[4] Gronemeyer, H. and Laudet, V. (1995) Protein Profile 2, 1173–1308.
[5] Nolte, R.T., Wisely, G.B., Westin, S., Cobb, J.E., Lambert, M.H., Kurokawa, R., Rosenfeld, M.G., Willson, T.M., Glass, C.K. and Milburn, M.V. (1998) Nature 395, 137–143.
[6] Shiau, A.K., Barstad, D., Loria, P.M., Cheng, L., Kushner, P.J., Agard, D.A. and Greene, G.L. (1998) Cell 95, 927–937.
[7] Feng, W., Ribeiro, R.C., Wagner, R.L., Nguyen, H., Apriletti, J.W., Fletterick, R.J., Baxter, J.D., Kushner, P.J. and West, B.L. (1998) Science 280, 1747–1749.
[8] Darimont, B.D., Wagner, R.L., Apriletti, J.W., Stallcup, M.R., Kushner, P.J., Baxter, J.D., Fletterick, R.J. and Yamamoto, K.R. (1998) Genes Dev. 12, 3343–3356.
[9] Torchia, J., Glass, C. and Rosenfeld, M.G. (1998) Curr. Opin. Cell Biol. 10, 373.
[10] Moras, D. and Gronemeyer, H. (1998) Curr. Opin. Cell Biol. 10, 384–391.
[11] Wolffe, P.A. (1997) Nature 387, 16–17.
[12] Kadonaga, J.T. (1998) Cell 92, 307–313.
[13] Struhl, K. (1998) Genes Dev. 12, 599–606.
[14] Chen, H., Lin, R.J., Schiltz, R.L., Chakravarti, D., Nash, A., Nagy, L., Privalsky, M.L., Nakatani, Y. and Evans, R.M. (1997) Cell 90, 569–580.
[15] Yao, T.-P., Ku, G., Zhou, R., Scully, R. and Livingston, D.M. (1996) Proc. Natl. Acad. Sci. USA 93, 10626–10631.
[16] Korzus, E., Torchia, J., Rose, D.W., Xu, L., Kurokawa, R., McInerney, E.M., Mullen, T.M., Glass, C.K. and Rosenfeld, M.G. (1998) Science 279, 703–707.
[17] Swope, D.L., Mueller, C.L. and Chrivia, J.C. (1996) J. Biol. Chem. 271, 28138–28145.
[18] Chen, H., Lin, R.J., Xie, W., Wilpitz, D. and Evans, R.M. (1999) Cell 98, 675–686.
[19] Wong, J., Shi, Y-B. and Wolffe, A.P. (1997) EMBO J. 16, 3158–3171.
[20] Freedman, L.P. (1999) Cell 97, 5–8.
[21] Voegel, J.J., Heine, M.J., Tini, M., Vivat, V., Chambon, P. and Gronemeyer, H. (1998) EMBO J. 17, 507–519.
[22] Jantzen, H.M., Strähle, U., Gloss, B., Stewart, F., Schmid, W., Boshart, M., Miksicek, R. and Schütz, G. (1987) Cell 49, 29–38.
[23] Strähle, U., Schmid, W. and Schütz, G. (1988) EMBO J. 7, 3389–3395.
[24] Hashimoto, S., Schmid, W. and Schütz, G. (1984) Proc. Natl. Acad. Sci. USA 81, 6637–6641.
[25] Schmid, E., Schmid, W., Jantzen, M., Mayer, D., Jastorff, B. and Schütz, G. (1987) Eur. J. Biochem. 165, 499–506.

[26] Weih, F., Stewart, A.F., Boshart, M., Nitsch, D. and Schutz, G. (1990) Genes Dev. 4, 1437–1449.
[27] Boshart, M., Weih, F., Schmidt, A., Fournier, R.E. and Schütz, G. (1990) Cell 61, 905–916.
[28] Nichols, M., Weih, F., Schmid, W., DeVack, C., Kowenz-Leutz, E., Luckow, B., Boshart, M. and Schütz, G. (1992) EMBO J. 11, 3337–3346.
[29] Nitsch, D., Boshart, M. and Schütz, G. (1993) Proc. Natl. Acad. Sci. USA 90, 5479–5483.
[30] Ganss, R., Weih, F. and Schütz, G. (1994) Mol. Endocrinol. 8, 895–903.
[31] Reik, A., Stewart, A.F. and Schütz, G. (1994) Mol. Endocrinol. 8, 490–497.
[32] Oshima, H. and Simons Jr., S.S. (1992) Endocrinology 130, 2106–2112.
[33] Simons Jr., S.S., Oshima, H. and Szapary, D. (1992) J. Steroid Biochem. Mol. Biol. 43, 43–55.
[34] Szapary, D., Oshima, H. and Simons Jr., S.S. (1992) Endocrinology 130, 3492–3502.
[35] Simons Jr., S.S., Oshima, H. and Szapary, D. (1992) Mol. Endocrinol. 6, 995–1002.
[36] Oshima, H. and Simons Jr., S.S. (1992) Mol. Endocrinol. 6, 416–428.
[37] Oshima, H. and Simons Jr., S.S. (1993) J. Biol. Chem. 268, 26858–26865.
[38] Oshima, H., Szapary, D. and Simons Jr., S.S. (1995) J. Biol. Chem. 270, 21893–21901.
[39] Zeng, H., Jackson, D.A., Oshima, H. and Simons Jr., S.S. (1998) J. Biol. Chem. 273, 17756–17762.
[40] Le Douarin, B., Zechel, C., Garnier, J.M., Lutz, Y., Tora, L., Pierrat, P., Heery, D., Gronemeyer, H., Chambon, P. and Losson, R. (1995) EMBO J. 14, 2020–2033.
[41] Fields, S. and Sternglanz, R. (1994) Trends Genet. 10, 286–292.
[42] Green, S., Issemann, I. and Scheer, E. (1998) Nucleic Acids Res. 16, 369.
[43] vom Baur, E., Zechel, C., Heery, D., Heine, M.J., Garnier, J.M., Vivat, V., Le Douarin, B., Gronemeyer, H., Chambon, P. and Losson, R. (1996) EMBO J. 15, 110–124.
[44] Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. (1990) J. Mol. Biol. 215, 403–410.
[45] Thompson, J.D., Higgins, D.J. and Gibson, T.J. (1994) Nucleic Acids Res. 22, 4673–4680.
[46] Gross, C.T. and McGinnis, W. (1996) EMBO J. 15, 1961–1970.
[47] Huggenvik, J.I., Michelson, R.J., Collard, M.W., Ziemba, A.J., Guerly, P. and Mowen, K.A. (1998) Mol. Endocrinol. 12, 1619–1639.
[48] LeBoeuf, R;D., Ban, E.M., Green, M.M., Stone, A.S., Propst, S.M., Blalock, J.E. and Tauber, J.D. (1998) J. Biol. Chem. 273, 361–368.
[49] Bloch, D.B., de la Monte, S.M., Guigaouri, P., Filippov, A. and Block, K.D. (1996) J. Biol. Chem. 271, 29198–29204.
[50] Dent, A.L., Yewdell, J., Puvion-Dutilleul, F., Koken, M.H., de The, H. and Staudt, L.M. (1996) Blood 88, 1423–1426.
[51] Kadereit, S., Gewert, D.R., Galabru, J., Hovanessian, A.G. and Meurs, E.F. (1993) J. Biol. Chem. 268, 24432–24441.
[52] Wilson, R., Ainscough, R., Anderson, K., Baynes, C., Berks, M., Bonfield, J., Burton, J., Connell, M., Copsey, T. and Cooper, J. et al. (1994) Nature 368, 32–38.
[53] Mattaj, I.W. (1989) Cell 57, 1–3.
[54] Miller, J., McLachlann, A.D. and Klug, A. (1985) EMBO J. 4, 1609–1614.
[55] Freedman, L.P., Luisi, B.F., Korszun, Z.R., Basavappa, R., Sigler, B.P. and Yamamoto, K.R. (1988) Nature 334, 543–546.
[56] Meyer, M.E., Gronemeyer, H., Turcotte, B., Bocquel, M.T., Tasset, D. and Chambon, P. (1989) Cell 57, 433–442.
[57] Bocquel, M.T., Kumar, V., Stricker, C., Chambon, P. and Gronemeyer, H. (1989) Nucleic Acids Res. 17, 2581–2595.
[58] Tasset, D., Tora, L., Fromental, C., Scheer, E. and Chambon, P. (1990) Cell 62, 1177–1187.
[59] Gill, G. and Ptashne, M. (1988) Nature 334, 721–724.
[60] Theriault, J.R., Charette, S.J., Lambert, H. and Landry, J. (1999) FEBS Lett. 452, 170–176.
[61] Christensen, J., Cotmore, S.F. and Tartesall, P. (1999) Mol. Cell. Biol. 19, 7741–7750.