The 21-base pair glucocorticoid modulatory element (GME) of the rat liver tyrosine aminotransferase (TAT)\(^1\) gene has been a useful model for steroid-induced gene expression over the years for several reasons. It is a biologically relevant response. It was one of the first systems to show a correlation between the steroid binding of receptors and whole cell induction of a protein (1). TAT induction is a primary effect of the receptor-steroid complex in that the induction of enzyme requires mRNA synthesis and is down-regulated by the removal of steroid (2). Finally, the TAT gene was shown to contain specific DNA sequences, called glucocorticoid response elements (GREs), which are steroid-inducible enhancers. GREs are obligatory components in the formation of the ternary DNA-receptor-steroid complexes that, in turn, are believed to interact with the transcriptional machinery to increase the rate of TAT gene transcription (3, 4).

Over the last few years, however, the picture has become much more complicated. Whereas the TAT GRE is still a “simple GRE” in that receptor-steroid complex binding to the isolated GRE is capable of inducing transcription without the help of other cis-acting elements or transcription factors (5), differences with other GREs have emerged, and many other TAT gene elements have been found to participate in the induction process. Thus, the TAT GREs are much further upstream from the start of transcription than for most other steroid hormone responsive elements (3, 6), and the transcription factor binding to the second GRE appears to be HNF-3 as opposed to the glucocorticoid receptor (7, 8). The region around the GREs of TAT extends from −2.6 to −2.3 kb has been found to be necessary for tissue-specific induction and has been called a glucocorticoid-responsive unit (6). In addition to the GRE, the glucocorticoid-responsive unit contains binding sites for the family of C/EBP proteins (9, 10) and an Ets-related factor (11) and harbors an element for tissue-selective gene expression (12). Tissue-specific elements are located at −3.6 kb, which binds CREB (7, 13, 14), at −5.5 kb (6, 9), at −11 kb (7, 15), and possibly at −350 to +1 bp (16, 17). A tissue nonspecific element that affects the level of induced activity has been localized at −3.0 to −2.6 kb (12).

All of the above TAT gene elements have been defined in the context of how, and when, glucocorticoid receptor activates or represses gene transcription in the presence of saturating concentrations of steroid. Arguably even more important for the functioning of intact cells are the responses with subsaturating concentrations of glucocorticoids because the intact cell or organism rarely is exposed to micromolar concentrations of glucocorticoid. Whereas those mechanisms regulating the level of response to saturating concentrations of agonist steroids should persist at subsaturating concentrations, thereby leaving the EC\(_{50}\) of the dose-response curve unaffected, the converse is...
not necessarily true. In fact, we have reported that the dose-response curve for TAT gene induction in Fu5-5 cells is left-shifted (to give a lower EC_{50}) relative to the same gene in HTC cells (18–21) or to a different gene in the same Fu5-5 cells (19, 21–24). Thus, physiological concentrations of glucocorticoid elicited a greater percentage of the maximal induction of the TAT gene in Fu5-5 cells than of any other genes examined. Although the fold change in the percent of maximal activity seen with subsaturating concentrations of glucocorticoid may seem small (e.g., 60% for the TAT gene versus 30% for other glucocorticoid-regulated genes), it is more than sufficient to permit differential control of gene expression by the same subsaturating concentration of glucocorticoid that a cell would see during development, differentiation, and homeostasis.

Another relatively unexplored area of steroid hormone action concerns anti-steroids, which block the action of agonists and thus have clinical utility. In parallel with the above studies of TAT dose-response curves, we observed that the amount of agonist activity displayed by anti-glucocorticoids such as dexamethasone 21-mesyate was much greater for TAT gene induction in Fu5-5 cells than in HTC cells (19, 21, 22).

Both the left shift in the dose-response curve (18, 19, 22–25) and the increased agonist activity of anti-steroids (19, 23, 24, 26) could be reproduced completely in the context of transiently transfected cells by a synthetic reporter gene (GREtkCAT) containing a 21-bp sequence (located at 3.6 kb of the TAT gene) that was positioned 5′ of the GRE. Furthermore, synthetic reporter constructs containing the 21-bp TAT sequence mimicked the endogenous TAT gene in the two other properties that have been examined: control of expression at the level of correctly initiated transcripts (26, 27) and response to changes in cell density (26–28). Thus, the properties of the endogenous TAT gene were faithfully reproduced in synthetic reporter constructs containing a GRE and the 21-bp element. To reflect its activity, we have called this 21-bp sequence a glucocorticoid modulatory element, or GME (25–29).

The characteristics of this GME sequence appear to be unique among those elements previously documented to participate in the transcriptional activation by steroid receptors. A cis-acting element of the distal promoter of the rat progesterone receptor gene has been described to cause just the opposite effects of this progesterone receptor gene (30). Thus, it is almost certain that different factors will be found to be responsible for the opposite effects of this progesterone receptor element and the GME.

Two proteins of 88 and 67 kDa that bind to the GME have been purified by oligo-affinity chromatography. The combination of these two proteins was sufficient to give a complex with the GME oligonucleotide in gel shift assays that was indistinguishable from that of the endogenous, cellular proteins (31). In both cases, biologically active, but not mutant inactive, GME oligonucleotide was able to inhibit the formation of a protein-DNA complex with 32P-GME oligonucleotide (26, 31). For those amino acids such as leucine and serine, for each of which there are six possible codons, the degeneracy of the synthetic oligonucleotides was reduced by using only those codons that are most commonly used in rat. Each degenerate oligonucleotide contained the same last two 3′ codons that are most commonly used in rat. Each degenerate oligonucleotide contained the same last two 3′ nucleotides to act as a “clamp” for hybridization, thereby increasing the frequency of productive elongation, with the cDNAs that were prepared by reverse transcription from the poly(A)-enriched rat liver Poly(A)+ RNA (27). The clones 21 and 22, which contained Poly(A)+ RNA isolated from total Fu5-5 RNA using either an oligo(dT) mRNA kit (Qiagen, Chatsworth, CA; for degenerate primer cloning) or two passes over oligo(dT)-cellulose spin columns (5′ Prime/3′ Rapid, Beverly, MA) and Promega.

**EXPERIMENTAL PROCEDURES**

Unless otherwise indicated, all operations were performed at 0 °C.

**Materials**—The following chemicals were purchased from the indicated sources: [32]Met, and oligonucleotide, Life Technologies, Inc.; acrylamide, bioacrylamide, and pretoained molecular weight markers, Bio-Rad and Life Technologies, Inc.; megaprimer RNA isolation kit, Stratagene (La Jolla, CA); reverse transcriptase-PCR system kit and TNT-coupled reticuloocyte lystate system, Promega (Madison, WI); restriction enzymes and DNA polymerase, New England Biolabs (Beverly, MA) and Promega.

**Buffers**—LSB buffer contained 20 mM HEPES (pH 7.9 at r.t.), 2 mM MgCl2, 0.5 mM dithiothreitol, 50 mM p-aminoethylbenzenesulfonyl fluoride, 10% glycerol, 0.2 mM EDTA, and 20 mM NaCl. Tris-buffered saline (TBS) had 20 mM Tris and 0.28 M NaCl in water (pH 7.5 at r.t.). The 2× SDS sample buffer contained 0.6× Tris (pH 8.8 at r.t.), 0.2 mM dithiothreitol, 2% SDS, 20% glycerol, and bromphenol blue. Western blot transfer buffer was comprised of 25 mM Tris (pH 8.3 at r.t.), 192 mM glycine, and 20% methanol.

**Cell Culture and Preparation of Cytosol**—Rat hepatoma tissue culture cells (clone 27 of Fu5-5 cells) were grown at 37 °C in a humidified incubator (5% CO2) in Richter's improved minimum essential medium with 10% fetal calf serum (Biofluidics, Rockville, MD). Cytosol was prepared as usual by freeze-thaw lysis and ultracentrifugation (32).

**Antibody**—A polyclonal rabbit antibody against the GMEB-2 sequence of ISPKEVFHLAGKSTLKDWKRAIR was prepared and affinity purified against the immunizing peptide by Zymed Laboratories (San Francisco, CA).

**Cloning of GMEB-2 cDNAs**—The rat GMEB-2 was cloned by preparing degenerate oligonucleotides of 20 bp in length for the three published tryptic fragments of GMEB-2 (31). For those amino acids such as leucine and serine, for each of which there are six possible codons, the degeneracy of the synthetic oligonucleotides was reduced by using only those codons that are most commonly used in rat. Each degenerate oligonucleotide contained the same last two 3′ nucleotides to act as a “clamp” for hybridization, thereby increasing the frequency of productive elongation, with the cDNAs that were prepared by reverse transcription from the poly(A)-enriched rat liver Poly(A)+ RNA (32). The clones 21 and 22, which contained Poly(A)+ RNA isolated from total Fu5-5 RNA using either an oligo(dT) mRNA kit (Qiagen, Chatsworth, CA; for degenerate primer cloning) or two passes over oligo(dT)-cellulose spin columns (5′ Prime/3′ Rapid, Beverly, MA) and Promega.

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After using all possible combinations of the primers for the three original tryptic fragments and a second round of PCR using a second primer that was 3′ of the first primer, a 278-bp cDNA was obtained which contained the appropriate portions of the peptides IMDSVELDNYFQIDK and AGLL-C77VQ7FFQHYEETMK at the 5′ and 3′ ends, respectively. This 278-bp cDNA was then used with the method of 5′- and 3′-rapid amplification of cDNA ends (RACE). Ligation-mediated 5′-RACE to generate a unique 5′ sequence (0.8 kb) was performed essentially according to the manufacturer’s recommendations using the CLONTECH 5′-RACE kit, cDNA prepared from poly(A)+ RNA (see above), prepoofreading DeepVent DNA polymerase (New England Biolabs), and two nested GMEB2-specific primers. Two 1.4-kb 3′ sequences, which overlapped the 5′ 0.8-kb sequence, were obtained using two nested GMEB2 gene-specific primers and a primer complementary to the anchor portion of the oligo(dt) primer used for cDNA synthesis (see above) with the native, prepoofreading form of Vent DNA polymerase (New England Bio- labs). The blunt-ended products of Vent and DeepVent synthesis were A-tailed with T4 polynucleotide polymerase according to the manufacturer’s recommendations (New England Biolabs) and cloned onto the T/A cloning vector, pCR2.0 (Invitrogen, Carlsbad, CA). Three 5′- and 3′-RACE clones were completely sequenced on both strands. The full-length cDNAs were obtained by joining the 5′ and 3′ fragments together after Apal cleavage in the overlapping region to give 2.0- and 1.9-kb clones.
To ensure that there were no PCR errors in this composite GMEB-2 sequence, an independent recloning of GMEB-2 was performed. A full-length 2.0-kb cDNA was recloned directly from Fu5-5 cell (clone 27) mRNA by standard reverse transcription and PCR amplification methods using oligonucleotides corresponding to 5'- and 3'-untranslated sequences of the composite GMEB-2. The 2.0-kb GMEB-2 cDNA was ligated into pCR2.1 (Invitrogen). Dideoxy sequencing (Sequenase 2.0 from U. S. Biochemical Corp.) of this direct GMEB-2 cDNA revealed no differences within the GMEB-2 coding region of the original GMEB-2 clone.

Isolation of Authentic GMEB-2—HTC cytosol was separated on 8% SDS-acrylamide gels, and the molecular mass range of 67 kDa, based on the migration of prestained molecular weight markers in adjacent lanes, was cut out of the gel and electroeluted in 1× SDS running buffer (25 mM Tris, 0.19 M glycine, 0.1% SDS) at 125 V in a model 1750 electroeluter (ISCO, Lincoln, NE) for 90 min at r.t. After elution, the protein was precipitated with 4 volumes of acetone (−60 °C). The pellets were redissolved in LSB buffer and reprecipitated with acetone as before. After the second precipitation, the recovered samples were denatured and renatured by redissolving in 100 μL of LSB buffer containing 6 M guanidinium hydrochloride and dialyzing against LSB buffer for 16 h. After dialysis, the renatured samples were concentrated to about 40 μL with a Microcon 10 concentrator and stored at −70 °C.

In Vitro Transcription and Translation—GMEB-2 protein was expressed in vitro from the clone in pCR2.1 with TNT T7-coupled reticulocyte lysate system (Promega) following the manufacturer’s suggestions.

Gel Shift Assays and Quantitation of Bands—The oligonucleotides 5'-CTTCTGTATGAGCGCCAGTAT-3' and 3'-GAAGACATACTCAGCGGTCT-5', which correspond to the GME of the rat TAT sequence at −3654 to −3634 bp from the start of transcription, were annealed and 32P-end-labeled by Lofstrand Laboratories (Gaithersburg, MD). Gel shift experiments were performed as described (26). Briefly, cytosol (0.5–2.0 μL) and the in vitro transcription/translation product (0.5–4.0 μL) were incubated with 20,000 cpm of the 32P-end-labeled GME (0.6 fmol) in a total volume of 20 μL with sheared, non-denatured herring sperm DNA (0.3 μg) as a nonspecific competitor. For immune-inhibition experiments, the antibody was added to the 50-μL reaction after the initial 20 min at 0 °C, and the incubation was continued for an additional 15 min at r.t. After electrophoresis in a 5% non-denaturing polyacrylamide gel at 150 V in 0.4× TBE, the dried gels were autoradiographed for 12–24 h at r.t. with Kodak X-Omat XAR-5 film. Alternatively, the gels were exposed to the phosphorimaging screen for the Molecular Dynamics Image-Quant system (Molecular Dynamics, Inc., San Diego, CA).

RESULTS

Cloning of GMEB-2—The rat GMEB-2 was cloned using the approach of PCR, with the primers being the degenerate oligonucleotides derived from the three tryptic fragments of GMEB-2 (31). After using all possible combinations of the three tryptic fragment primers, and a second round of PCR using internal nested primers, a 278-bp cDNA was obtained. 5'- and 3'-RACE were used to generate a unique 5' sequence (0.8 kb) with minor differences upstream of the open reading frame (see below), which overlapped with two 1.4-kb 3' sequences containing more major differences at the 3' ends (Fig. 1). Fusion of the two sets of fragments yielded two cDNA clones of 2.0 and 1.9 kb. Both clones encoded open reading frames (529 and 485 for the 2.0- and 1.9-kb clones, respectively) bounded by in frame stop codons and contained the three sequenced GMEB-2 peptides (31) (Fig. 2). These results argue that both clones represent full-length clones and that the smaller 1.9-kb clone originated from alternative splicing of the precursor of the longer 2.0-kb mRNA transcript. A fourth tryptic peptide of the original 67-kDa protein was sequenced (Keck Foundation, Yale University) in hopes of obtaining a fragment that was unique to
one of the two 3' sequences, thereby permitting a direct identification of the cDNA for the isolated protein. Unfortunately, the fourth peptide (XXVLLNNIVONFGMLDLVK) was also common to both 3' clones, and no other tryptic fragment of the GMEB-2 protein (31) was suitable for further sequencing.

To ensure that there were no PCR errors in the originally obtained GMEB-2 sequence, an independent recloning of GMEB-2 was carried out. Based on the known 5' - and 3' -nontranslated sequences of GMEB-2, the full-length 2.0-kb cDNA was recloned directly from Fu5-5 clone 27 cellular mRNA by the reverse transcription and PCR amplification method. No sequence disagreements within the GMEB-2 coding region were observed between the above two independent 2.0-kb GMEB-2 clones as determined by DNA sequencing with Sequenase 2.0. Thus, this appears to be the correct sequence for the rat protein.

This protein is predicted to be an acidic protein with a pI of 5.0. This protein appears to be a novel protein. Routine BLAST searches of GenBank™ have yet to reveal any other protein that is identical or even homologous to that encoded by either clone (last search was 2/11/98).

Biochemical Properties of GMEB-2—The predicted molecular weight of both suspected GMEB-2 clones was much less than that expected. The 2.0-kb cDNA encoded protein has a calculated molecular mass of 56,535 Da versus the expected 67 kDa, whereas the calculated size for the 1.9-kb cDNA product was 55,799 Da.
Cloning of Rat GMEB-2

**Western blotting.** Samples of HTC cell cytosol, in vitro translated GMEB-2 (programed lysate), or reticulocyte lysate that had been incubated with luciferase cDNA as carrier DNA (unprogramed lysate) were separated on 10% SDS-polyacrylamide gels and transferred to nitrocellulose for Western blotting with affinity purified anti-GMEB-2 antibody in the absence (w/o Peptide) or presence (Peptide) of antigenic peptide (4 μg/ml). The arrow indicates the position of GMEB-2. The ECL background in the presence of the antigenic peptide was very high, presumably due to a high binding affinity of the peptide to the nitrocellulose filter. In order to block the nonspecific binding of peptide to filter, 10% non-fat dried milk (Carnation) was included in all solutions from the addition of peptide to the addition of secondary antibody.

![Image](97x527 to 249x729)

**Fig. 4. Detection of authentic and in vitro translated GMEB-2 by Western blotting.** Samples of HTC cell cytosol, in vitro translated GMEB-2 (programed lysate), or reticulocyte lysate that had been incubated with luciferase cDNA as carrier DNA (unprogramed lysate) were separated on 10% SDS-polyacrylamide gels and transferred to nitrocellulose for Western blotting with affinity purified anti-GMEB-2 antibody in the absence (w/o Peptide) or presence (Peptide) of antigenic peptide (4 μg/ml). The arrow indicates the position of GMEB-2. The ECL background in the presence of the antigenic peptide was very high, presumably due to a high binding affinity of the peptide to the nitrocellulose filter. In order to block the nonspecific binding of peptide to filter, 10% non-fat dried milk (Carnation) was included in all solutions from the addition of peptide to the addition of secondary antibody.

![Image](321x417 to 541x729)

**Fig. 5. Binding of in vitro translated GMEB-2 to GME oligonucleotide in gel shift assay.** 32P-GME oligonucleotide was incubated with HTC cell cytosol, unprogramed lysate, or various combinations of programed lysate or gel purified GMEB-1 or -2 from HTC cell cytosol. A, comparison of complex formation with GME of in vitro translated GMEB-2 (→), unprogramed lysate, and HTC cell cytosol containing GMEB-1 and 2. B, synergistic binding of authentic, or in vitro translated, GMEB-2 and authentic GMEB-1 to GME oligonucleotide. Authentic GMEB-1 and -2 were separated on SDS-polyacrylamide gels, extracted, and individually renatured overnight as described (31). The various combinations of proteins were incubated with labeled DNA overnight and then analyzed as described under “Experimental Procedures.” The position of the authentic complex of GMEB-1 and -2 with 32P-GME oligonucleotide is indicated by the arrow.

(Fig. 5A). This binding was competed by excess GME oligonucleotide but not by the biologically inactive oligonucleotide M2 (26), thus showing that the binding of the in vitro translated GMEB-2 is sequence-specific.

Authentic GMEB-1 and -2 were then isolated from HTC cell cytosol. As shown in Fig. 5B, the gel-shifted complex with recombinant GMEB-2 migrated slightly faster than that of both the endogenous GMEB-1 and -2 complex from HTC cell cytosol (lanes 6 versus 7) and the reconstituted complex with recombinant GMEB-2 and gel-purified GMEB-1 (lanes 6 versus 5). Furthermore, the combination of recombinant GMEB-2 and authentic GMEB-1 synergized to give more gel-shifted complex than the sum of the two individual components (lanes 5 versus 2 and 6).

Finally, anti-GMEB-2 antibody could reduce the amount of gel-shifted complex formed between the GME oligonucleotide and GMEB-2 (compare lane 5 versus lane 4 in Fig. 6, GMEB-2 complex is indicated by the arrow). However, a 15-min incubation at r.t. was required to see the immuno-disruption of the GME-GMEB-2 complexes, consistent with the inability of anti-GMEB-2 antibody to immunoprecipitate GMEB-2 from solution at 0 °C. It is of interest that the same antibody treatment

\[^{2}\] S. Kaul and S. S. Simons, manuscript in preparation.
Cloning of Rat GMEB-2

DISCUSSION

We had previously reported that the 67-kDa GMEB-2 is part of a heteromeric complex of two new proteins that bind to a cis-acting GME element of the TAT gene (31) in a manner that appears to modulate the activity of glucocorticoid receptors bound to a GRE (23–26, 29). In view of the unusual properties of the GME, which causes a left shift in the dose-response curve of GR-agonist complexes and increased amounts of agonist level of complex containing GMEB-1 and -2 (lanes 2 versus 3 and lanes 4 versus 5). The lower levels of this unknown species in the immune serum was probably a result of the partial purification of the anti-GMEB antibody.

at r.t. was unable to reduce the amount of complex formed with the GMEB-1/-2 heteromer (lanes 3 versus 2), perhaps indicating a tighter association of the heteromeric complex, which is reflected in the approximately equal amount of heteromeric complex that was formed from HTC cell cytosols and in vitro translated GMEB-2 (lanes 2 and 4 of Fig. 6) even though HTC cell cytosol contains less GMEB-2 (see Fig. 4). In three separate experiments, the immuno-depletion of the GMEB-2 complexes (final = 65 ± 10%, S.D.) was significantly greater than that of the GMEB-1/-2 complexes from HTC cells (final = 108 ± 7%, S.D., p < 0.0013). Therefore, by several criteria, the 2.0-kb clone that we have isolated encodes a novel protein that corresponds to the 67-kDa GMEB-2.

It should be noted that preimmune serum had no effect on the level of complex containing GMEB-1 and -2 (lanes 2 versus 3). However, both preimmune and immune sera contained an uncharacterized species that afforded a much more slowly migrating complex (lanes 2 versus 3 and lanes 4 versus 5). The lower levels of this unknown species in the immune serum was probably a result of the partial purification of the anti-GMEB antibody.

Many transcription factors, such as the steroid receptors (37), NF-κB/Rel (38), and Jun/Fos/CREB (39), are members of a large superfamily of related proteins. We were therefore surprised to find that there were no large regions of GMEB-2 that were homologous to anything in GenBank™. A family of proteins that interacts with steroid receptors has recently been found to be composed of SRC-1, TIF2, pCIP, ACTR, RAC3, and AIB1 (40), each of which contains a variety of shared motifs such as basic helix-loop-helix/PAS, serine/threonine-rich, glu-

32P-GME oligonucleotide was incubated with GMEB-1/-2 from HTC cell cytosol, followed by buffer, preimmune serum, or non-purified anti-GMEB antibody. Similarly, 32P-GME oligonucleotide was incubated with in vitro translated GMEB-2, followed by preimmune serum or non-purified anti-GMEB antibody. The migration of the GMEB-2 complex is indicated by the arrow.

FIG. 7. Homology between GMEB-2 and other proteins. Alignment of sequences of the open reading frames of four C. elegans cosmid, Drosophila DEAF-1, and rat and human Suppressin, with a region of GMEB-2. The alignment was performed by SeqApp, which ascribes different colors to various amino acids that are unrelated to the homology.

in vitro translation of the cDNA clone migrated as a 67-kDa protein on SDS gels. Furthermore, Western blotting with an antibody raised against a non-sequenced peptide of the GMEB-2 indicated that the synthetic protein co-migrated with a 67-kDa protein in HTC cell cytosol, which is known to contain GMEB-2 (31). This Western blotting of endogenous and in vitro translated GMEB-2 was selectively blocked by the presence of excess antiserum. Finally, the recombinant GMEB-2 protein formed a sequence-specific gel-shifted complex with a GME oligonucleotide that could be inhibited by anti-GMEB-2 antibody and displayed the same gel shift properties as did authentic GMEB-2. Thus, we conclude that the cDNA that we have cloned does encode the rat GMEB-2 protein.

The difference between predicted size of GMEB-2 and that observed on SDS gels was unusually large. However, the discrepancy does not appear to result from posttranslational modifications as the DNA binding and oligomerization of material prepared by in vitro translation are the same as for the GMEB-2 from cells. Although further studies are required to confirm this, we suspect that the aberrant migration on SDS gels is due to the presence of some sequence, just as has been identified for GR, which migrates as a protein that is about 10 kDa larger than its predicted size (34).

A demonstration of the biological activity, and relevance, of GMEB-2 awaits the cloning and characterization of the hetero-oligomeric partner, GMEB-1 (31). However, GMEB-2 binding to the GME oligonucleotide is prevented only by biologically active mutant oligonucleotides (26), and GMEB-2 does have some intrinsic transcriptional activity in mammalian cells. The entire genome of several prokaryotic organisms has recently been cloned (Ref. 35 and references therein). The fact that none of these genomes contain sequences that are highly homologous to GMEB-2, as determined by a tBLASTN search, suggests that GMEB-2 is an evolutionarily recent protein. This conclusion is in keeping with the apparent role of GMEB-2 in modulating the activity of glucocorticoid receptor-regulated gene expression (18–22, 25–29).

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tamine-rich, and CBP-interacting domains (41–43). These pro-
teins also interact with the receptors via domains that contain
the small sequences of LXXLL (40, 41, 44). GMEB-2 does
contain both serine/threonine-rich (32 and 26% in sequences
171-232 and 336–516, respectively) and glutamine-rich (17%
among amino acids 250–321) domains, but their functional
significance is not yet known. No basic helix-loop-helix/PAS,
CBP-interacting, or receptor-interacting domain with homol-
dy to those of SRC-1, TIF2, pCIP, ACTR, RAC3, and AIB1
were found (data not shown). However, one local homology
was noted with another selection of proteins. GMEB-2 contains a
KDWKR sequence which has recently been reported in the
DEAF-1 protein from Drosophila (45), rat Supressin (46, 47),
three human EST clones, and a Caenorhabditis elegans cosmids.
We have found essentially the same sequence in rat and hu-
an.3 Suppressin, DEAF-1, and three other C. elegans cosmids
(Fig. 7). Both Supressin and DEAF-1 are transcription factors
with interesting properties. Supressin is a 63-kDa protein
that has all the characteristics of a global negative regulator of
cell proliferation and especially the immune system. Supressin
arrests lymphocytes in the G0/G1 phase of the cell cycle af-
fter reduction of their RNA, protein, and DNA synthesis, sug-
uggesting that Supressin inhibits the processes required for
G0 transition to G1. DEAF-1, an 85-kDa protein, binds to a
specific DNA region of the 120-bp homeotic response element
that is regulated specifically by the Deformed gene product in
Drosophila embryos. DEAF-1 and its element are required for
the functional activity of the 120-bp Deformed response ele-
ment and thus is functionally a cofactor. Among these known
and putative proteins, a consensus sequence of (S/T)P-(E/Q)F—
(K/R)—KDWK-(I/R)K has emerged that is slightly dif-
derent than that noted by others (45). These data suggest that
the novel 529-amino acid protein GMEB-2 is a potentially nu-
tritional transcription factor which might belong to a new transcrip-
tion factor family that includes DEAF-1 and Supressin. It is in-
teresting to note that one tryptic peptide of GMEB-1, FVHLAGK
(31), is identical to the sequence of GMEB-2 within this con-
sensus sequence and thus may be another member of this
potential larger family.

The function of the consensus sequence of (S/T)P-(E/Q)F—
(K/R)—KDWK-(I/R)K is not known. However, the fact that an
antibody raised against this sequence could not immuno-
precipitate GMEB-2 but was very sensitive in recognizing
an antibody raised against this sequence could not immuno-
precipitate GMEB-2 but was very sensitive in recognizing
a peptide containing the (K/R)- - -KDWK–I(R/K) is not known. However, the fact that
the functional activity of the 120-bp Deformed response ele-
ment is identical up to nucleotide 1520. A

3 R. D. LeBoeuf and J. D. Tauber, unpublished results.