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# Characterization of a hepatoma mRNA transcribed from a third promoter of a 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-encoding gene and controlled by *ets* oncogene-related products

**(fetal/fructose 2,6-bisphosphate/GA binding protein/transcription factor Sp1/TATA-less promoters)**

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**ABSTRACT** 6-Phosphofructo-2-kinase (EC 2.7.1.105)/fructose-2,6-bis-phosphatase (EC 3.1.3.46) catalyzes the synthesis and degradation of fructose 2,6-bisphosphate, a ubiquitous stimulator of glycolysis. The liver (L-type) and muscle (M-type) mRNAs for this bifunctional enzyme arise from distinct promoters of the same gene. We have now characterized in rat hepatoma FTO2B cells another mRNA, which is transcribed from a third promoter of that gene. This F-type mRNA is present in fetal rat liver and muscle, in rat placenta, and in several established rat cell lines. The F promoter contains no TATA box but contains several binding sites for Sp1 and for members of the *ets* oncogene family. Transfection of FTO2B cells with constructs containing the intact or mutagenized F promoter showed that its activity depends mainly on one of these sites. This site bound a heteromeric FTO2B cell protein indistinguishable from the *ets*-related GA binding protein $\alpha$/ankyrin-repeats GA binding protein $\beta$ transcription factor.

In many types of transformed cells the glycolytic flux is abnormally high, even in aerobiosis (1). This situation may involve fructose 2,6-bisphosphate, a potent stimulator of 6-phosphofructo-1-kinase (2). The synthesis and degradation of fructose 2,6-bisphosphate are catalyzed by the bifunctional enzyme 6-phosphofructo-2-kinase (PFK-2)(ATP: D-fructose-6-phosphate 2-phosphotransferase, EC 2.7.1.105)/fructose-2,6-bisphosphatase (FBPase-2) (D-fructose-2,6-bisphosphate 2-phosphohydrolase, EC 3.1.3.46). There are several PFK-2/FBPase-2 isozymes that differ in tissue distribution and control by protein kinases (3). We have cloned a 55-kb gene (gene A) that encodes the liver (L-type) and muscle (M-type) isozymes through use of different promoters (4). The L-type (2.1 kb) and M-type (1.9 kb) mRNAs share 13 consecutive exons. The 5′ end of each mRNA consists of an additional coding exon, which is specific for the M (exon $1_M$) or for the L (exon $1_L$) isozyme (5). In the gene, exon $1_L$ lies 4.3 kb downstream from exon $1_M$ (Fig. 1*A*). Thus, isozyme M has the same sequence as isozyme L, except for the N terminus where the first 32 residues of isozyme L are replaced by an unrelated nonapeptide. As a result, isozyme M is not controlled by cAMP-dependent protein kinase which, in isozyme L, phosphorylates Ser-32, thereby activating FBPase-2 and inactivating PFK-2.

Rat hepatoma PFK-2/FBPase-2 differs from isozyme L (7, 8). The mRNA from the FTO2B hepatoma cell line hybridizes with a probe specific for exon $1_M$ but does not hybridize with a probe specific for exon $1_L$, and yet it is 0.3 kb longer than the M-type mRNA (8). We have now cloned from FTO2B cells a PFK-2/FBPase-2 mRNA that we call F-type.§ It arises from gene A through use of a third promoter located 1.5 kb upstream from exon $1_M$. This F promoter has unique features when compared with the M and L promoters and is controlled by transcription factors of the *ets* family.

## MATERIALS AND METHODS

**Construction, PCR Amplification, and Screening of the FTO2B cDNA Library.** Total RNA was extracted (9) from FTO2B cells (6) exposed for 12 hr to 1 $\mu$M dexamethasone (8). Poly(A)-rich RNA (10) was used to construct a cDNA library in λZAPII (Stratagene). PCR was done with *Taq* DNA polymerase and buffers from Boehringer Mannheim on 1.7 × $10^7$ phages heated at 70°C for 10 min, using primers at 0.6–1.8 $\mu$g/ml and 30–40 cycles of 1 min at 50°C, then at 72°C, then at 95°C followed by a step of 10 min at 72°C. PCR products were ligated in a T-vector prepared from pBluescript (11). To screen the library, plaques (4 × $10^6$ phages) were replicated on Hybond-C-extra (Amersham) membranes prehybridized for 4 hr at 65°C in 6× standard saline/citrate (SSC) (12)/5× Denhardt's solution (12)/0.5% SDS/10 mM sodium phosphate, pH 6.9/1 mM EDTA. Hybridization with the Megaprime (Amersham)-labeled (3.$10^5$ cpm/ml) 1.4-kb 22c1 cDNA probe (5) lasted for 16 hr at 65°C in the same solution containing herring sperm DNA at 100 $\mu$g/ml and the probe denatured for 5 min at 100°C. The membranes were washed three times (5 min, room temperature) in 2× SSC/0.1% SDS and once (60 min, 65°C) in 0.5× SSC/0.1% SDS. pBluescript phagemids, obtained according to Stratagene from the selected λZAP phages, were used for sequencing with Sequenase (United States Biochemical) and with T3 and T7 primers and oligonucleotides (oligos) made by Pharmacia.

**DNA and RNA Analysis.** For Southern blotting (12) oligo G (Fig. 1*B*) was 5′ end-labeled with [$\gamma$-$^{32}$P]ATP and T4 polynucleotide kinase (New England Biolabs). For RNA blotting, poly(A)-rich RNA (10) was denatured, electrophoresed, and hybridized as described (13). For RNase protection (14), antisense RNA probes were hybridized (16 hr, 46°C) with 25

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Abbreviations: FBPase-2, fructose-2,6-bisphosphatase; oligo(s), oligonucleotide(s); PFK-2, 6-phosphofructo-2-kinase; GABP, GA binding protein.

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§The sequence reported in this paper has been deposited in the GenBank data base (accession no. M26215).

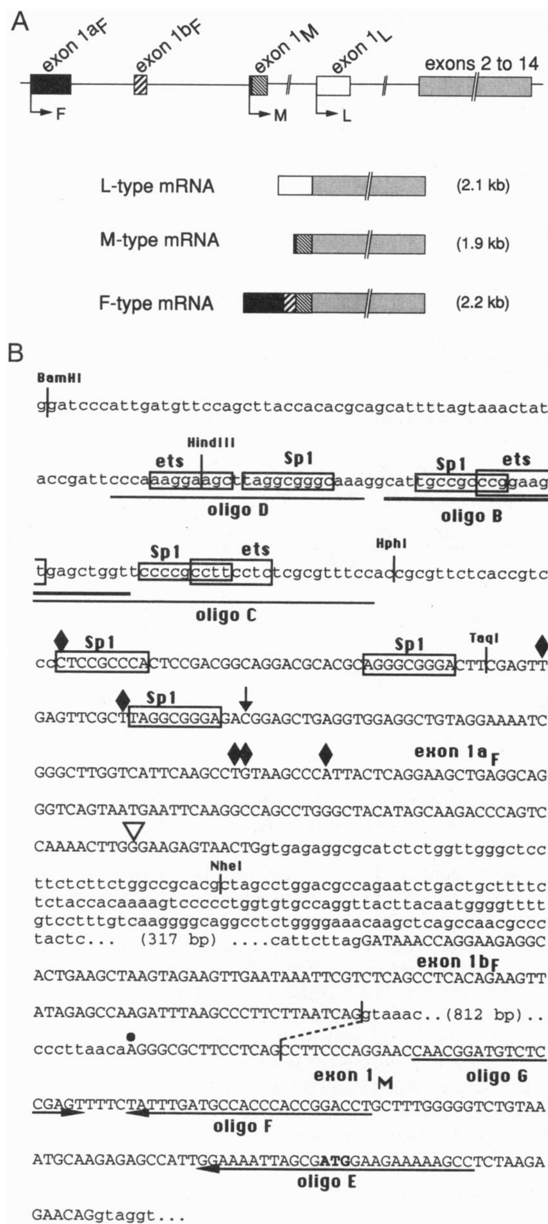


FIG. 1. (*A*) Organization of the 5′ end of PFK-2/FBPase-2-encoding gene A and of three mRNAs transcribed from that gene, with their transcription initiation sites (arrows). (*B*) Sequence of the F promoter and of the region of gene A that encodes the 5′ end of F-type mRNA. Uppercase letters correspond to exons. Intron sequences are not shown, except for the splice sites consensus and the part of the intron between exons $1a_F$ and $1b_F$ not published in refs. 4 and 6. ◆, 5′ ends of mRNA determined by RNase protection; ↓, 5′ end of cDNA; ▿, 5′ end of PCR product; ●, 5′ end of M-type mRNA; - - - between exons $1b_F$ and $1_M$, splicing as it occurs in F-type mRNA; ATG in boldface type, translation initiation site of isozyme M; boxes, consensus for the factors indicated. Oligos correspond to the sequences underlined, with an arrow pointing toward the 3′ end of the single-stranded oligos.

μg of total RNA or with 2 μg of poly(A)-rich RNA extracted directly from frozen tissues with the Dynabeads system (Dynal, Oslo) and mixed with 20 μg of yeast tRNA. The incubation was treated for 30 min at 30°C with RNase T1 (1.2–2.5 μg/ml) and RNase A (20–40 μg/ml), and the digestion products were analyzed on a 6% acrylamide sequencing gel.

**Plasmids, Site-Directed Mutagenesis, and Oligos.** The *Hph* I–*Taq* I and *Bam*HI–*Taq* I fragments (Fig. 1*B*) were cloned in pXP2 (6) upstream from the firefly luciferase reporter gene. Site-directed mutagenesis was done (Amersham Mutagenesis system version 2) on the *Bam*HI–*Taq* I fragment cloned in M13mp8, with the following antisense oligos in which the underlined nucleotides of the *ets* motifs had been modified as indicated: 5′-GAAACGCGAGACCAAGGCGGGGAAC (proximal motif), 5′-CCAGCTCACTTGGGGGCG-GCAATG (central motif), and 5′-GCCTAAGCTTGGTT-TGGGAATCG (distal motif). Double-stranded oligos B and C were described (6), and oligo D was as shown in Fig. 1*B*, with cohesive *Xba* I ends (CTAGA) added. Oligos Dmut*ets* and DmutSp1, with the nucleotides underlined modified as indicated, were 5′-CTAGACCCAAACCAAGCTTAG-GCGGGCAAA and 5′-CTAGACCCAAAGGAAGCTTAG-TATGGCAAA. Oligo *ets* was ATAACCAGGAAGTGGGC with 5′-end *Ava* I–*Xho* I sites and a 3′-end *Ava* I site. Oligo *ets*mut was oligo *ets* with the central GG replaced by CC. Oligo Sp1 was from Promega. The single-stranded oligos E, F, and G are shown in Fig. 1*B*.

**Cell Transfection and Gel-Shift Assays.** FTO2B cells were transfected (6), with pSV2CAT as internal control. Luciferase and chloramphenicol acetyltransferase were assayed with the Promega and Boehringer Mannheim kits, respectively. Gel shifts were done (6) with FTO2B nuclear extracts (15), with SDS/PAGE fractions of these extracts (16), or with recombinant rat GA binding protein (GABP) α and -β1, chicken *Ets1* (17), and Sp1 (18). Anti-Ets antiserum was described (19). Full-length rat GABPα and -β1 expressed in *Escherichia coli* and rabbit antisera against these proteins were provided by T. Brown and S. McKnight, Carnegie Institution of Washington, Baltimore.

## RESULTS AND DISCUSSION

**Structure and Expression of the F-type mRNA.** Exon $1_M$ contains a 3′ splice-site consensus (4). This fact and hybridization data (8) suggested that the F-type mRNA has specific 5′ terminal exon(s) spliced with exon $1_M$. The putative F-type-specific 5′ end was searched for in the FTO2B cDNA library by PCR between the T3 primer of λZAP and first oligo E, then oligo F (Fig. 1*B*). This procedure yielded products that hybridized by Southern blotting with oligo G, which were then cloned in pBluescript. These clones shared the same sequence from the 3′ end of the insert. Four of them extended upstream of exon $1_M$. The longest clone contained an exon $1_M$ lacking the first 15 bp but preceded by 100 nt found 842 bp upstream in gene A, themselves preceded by 13 nt found 510 bp more upstream in the gene (Fig. 1*B*). An RNA probe containing the first 113 nt on the 5′ side of the PCR product revealed, on RNA blots of FTO2B RNA, a 2.2-kb band which increased in intensity, as expected (8), in cells treated with dexamethasone (data not shown). Thus, FTO2B mRNA derives from gene A; it contains at least two exons that are not found in the M-type or L-type mRNA and are spliced with an alternative acceptor site in exon $1_M$ (Fig. 1*B*).

To obtain the full sequence of the mRNA, we screened the cDNA library with a cDNA probe (exons 3–14 of gene A) known to hybridize with FTO2B mRNA (8). Ten positive clones out of $4 \times 10^6$ recombinant clones were found. One clone contained a cDNA that extended from the poly(A) tail to include not only the PCR product but also 139 additional bp on the 5′ side. The other clones were incomplete, with a 5′ end located in exon $1_M$ or downstream from it. In gene A, the additional sequence of the longest clone is in direct continuation with that of the PCR product. To determine whether this cDNA was a full-length copy of the FTO2B mRNA, RNase protection was performed with an RNA probe corresponding to the *Bam*HI–*Nhe* I fragment (Fig. 1*B*), which extends 219 nt upstream from the cDNA. Five protected bands were observed with transcription initiation sites

located, within 120 bp, upstream and downstream from the 5′ end of the cDNA (Fig. 1*B*). The presence of multiple initiation sites was consistent with the promoter structure (see below). The sequence of the F-type mRNA was established from the longest clone. This sequence was identical to that of the M-type mRNA (5), except for the deletion of 15 nt at the 5′ end of exon $1_M$ and for the addition of two exons, termed exons $1a_F$ and $1b_F$, upstream from the truncated exon $1_M$ (Fig. 1*A*). When compared with the length of the M-type mRNA—i.e., 1708 nt excluding the poly(A) tail—the length of the F-type mRNA (2010 nt) was consistent with the difference of 0.3 kb seen between these two mRNAs by Northern blotting (8).

To determine tissue distribution of F-type mRNA, RNase protection was done with an RNA probe specific for this mRNA (Fig. 2). The mRNA was present in hepatoma, fibroblast, and myoblast cell lines. Its concentration decreased upon differentiation of L6 myoblasts into myocytes (Fig. 2) and in Rat-1 fibroblasts made quiescent by lowering serum concentration in the culture from 10 to 0.1% (data not shown). F-type mRNA concentration increased in FTO2B cells upon dexamethasone treatment, as expected (8). This mRNA was found in the two fetal tissues examined—namely, liver and muscle—and in lung and thymus. This mRNA was present at much lower levels or was undetectable in the other adult tissues tested. The highest concentration was in preterm placenta, with a decrease at term. Thus, expression of the F-type mRNA appears to correlate with cell proliferation.

Translation of the M-type mRNA starts in exon $1_M$ (21). In the F-type mRNA, there is one AUG upstream from this exon, but a stop codon is encountered eight triplets downstream. Therefore, exons $1a_F$ and $1b_F$ are noncoding, and the sequence of the protein translated from this mRNA is not expected to differ from that of isozyme M. Several other genes have distinct promoters that function in a stage- or tissue-specific manner and give rise to different mRNAs with the same coding sequence but different 5′ leaders (22). Our identification of the 5′ end of the F-type mRNA allowed characterization of the F promoter.

**Characterization of F Promoter.** A construct containing the *Bam*HI–*Taq* I genomic fragment (Fig. 1*B*) upstream from a promoterless luciferase reporter gene was transfected in FTO2B cells. This fragment behaved as a promoter: luciferase activity was stimulated 100- to 1000-fold the background (Fig. 3, construct a versus construct k). The 150 bp upstream from the 5′ end of the cDNA contain no TATA box but contain six Sp1 consensus (23) sequences (Fig. 1*B*); this is typical of TATA-less promoters, in which multiple cap

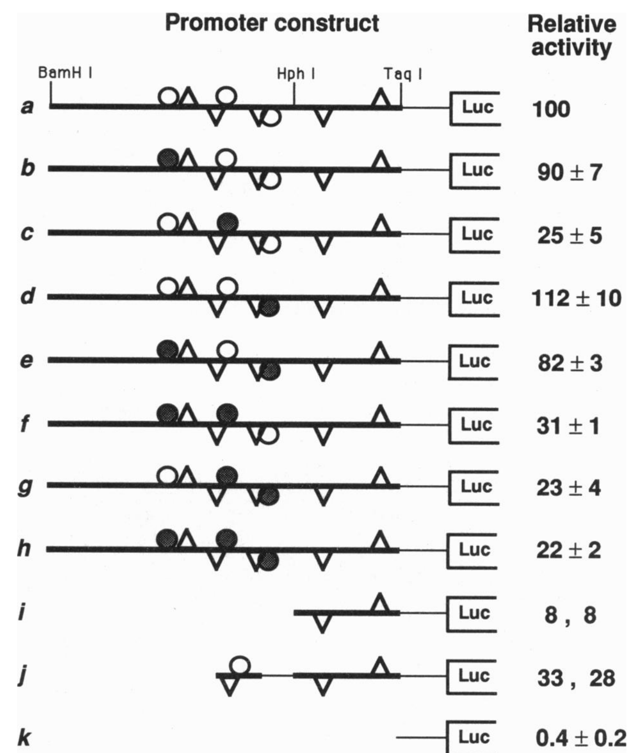


FIG. 3. Promoter activity and effect of site-directed mutagenesis of *ets* motifs. The *Bam*HI–*Taq* I or *Hph* I–*Taq* I fragment (thick bars and Fig. 1*B*) was cloned upstream from the promoterless luciferase (Luc) reporter gene and transfected in FTO2B cells. Thin bars are pXP2 polylinker sequences. Circles and triangles refer to the *ets* and Sp1 motifs, respectively. Consensus on the sense strand are shown by symbols above the line; consensus on the antisense strand are shown by symbols below the line. Filled circles refer to *ets* motifs destroyed by site-directed mutagenesis. Relative activities are ratios of luciferase activity of the test plasmid to chloramphenicol acetyltransferase activity of the cotransfected control plasmid, normalized for activity of the nonmutated promoter at 100; for the latter, absolute luciferase activity values ranged from 6,000 to 50,000 units. Data are means ± SEM for four or five independent experiments, except for constructs i and j (two experiments).

sites exist (24), as found here. This region also contains three binding motifs for transcription factors of the *ets* oncogene family (25, 26), which includes at least 12 members that share a conserved DNA-binding domain. Some of them are tissue-

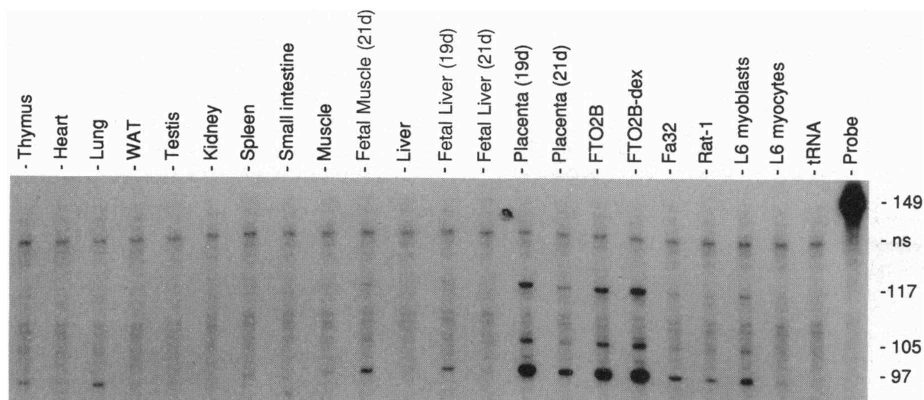


FIG. 2. Rat tissue distribution of F-type mRNA evaluated by RNase protection of poly(A)-rich RNA. WAT, white adipose tissue; Fa32, rat hepatoma cell line (20); Rat-1, rat fibroblast cell line (6); L6, rat myogenic cell line (6); d, days of gestation; dex, dexamethasone (1 $\mu$M for 12 hr). The 149-nt RNA probe contained the last 13 nt of exon $1a_F$ plus 100 nt of exon $1b_F$ and pBluescript polylinker sequences. Because the probe contained one PCR-generated mismatch located 20 nt from the 3′ end, protected fragments of 117 and 97 nt were expected. Protected fragments of 105 nt could correspond to unspliced transcripts. ns, Nonspecific (see tRNA control lane).

restricted or development-specific or are involved in the cell response to extracellular signals (27).

To assess the contribution of these *ets* motifs to F promoter activity, we changed to CC the core GG residues that specifically contact *ets* family members and are essential for their binding to DNA (26, 28, 29). Fig. 3 shows that loss of the distal (construct b) or of the proximal (construct d) *ets* motifs had no effect, whereas loss of both (construct e) decreased promoter activity by 15–20%. In contrast, destruction of the central *ets* motif (construct c) decreased promoter activity by 75%, an effect not strengthened by the concomitant loss of either one (constructs f or g) or of the two other (construct h) *ets* sites. About a third of the residual activity of the triple *ets* mutant was ascribed to the two Sp1 sites located downstream from the proximal *ets* site. Indeed, the *Hph* I–*Taq* I promoter fragment (construct i), which binds only Sp1 in FTO2B extracts (6), displayed <10% of the activity of the intact promoter. Addition to this fragment of the central *ets*/Sp1 cluster (oligo B) failed to restore promoter activity to the expected level (construct j versus e), suggesting that other cis-acting sequences, such as the Sp1 motifs present in the proximal and/or distal *ets*/Sp1 clusters, also contribute to F promoter activity. In any case, the central *ets* site accounted for most activity of the F promoter. This result was consistent with our findings (6) that the protein(s) specifically bound by the *Hin*dIII–*Hph* I fragment (Fig. 1*B*) in FTO2B extracts interact(s) exclusively with the central *ets* site and that the only DNase I footprint seen with such extracts on the whole promoter (*Bam*HI–*Taq* I fragment) corresponds to this site.

**Factor Binding to F Promoter.** This binding was investigated by performing gel shifts with oligos matching (Fig. 1*B*) the three *ets*/Sp1 clusters. Oligo B, which corresponds to the central cluster, produced with FTO2B extracts four specific shifts (bands a–d) that were inhibited by oligo *ets*, a high-affinity binding site for Ets1 and other members of the *ets* family, but not by oligo *ets*mut (Fig. 4, lanes 1–4). Oligo Sp1 did not compete (lane 11). Lack of binding of Sp1 in the extracts was not from hindrance by the *ets*-like proteins because recombinant Sp1 alone did not bind to oligo B (data not shown). Oligo C, which corresponds to the proximal cluster, did not compete either (lane 10). Instead, it produced with FTO2B extracts a specific shift that was inhibited by oligo Sp1, and it bound recombinant Sp1 (data not shown).

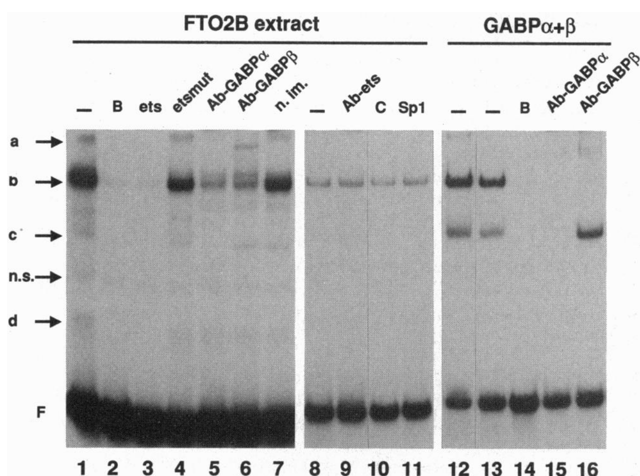


FIG. 4. Gel-shift assays of the central portion (oligo B, Fig. 1*B*) of the F promoter with the proteins indicated, in absence (lanes 1, 8, 12, 13) or presence of the competing oligos (30 ng) (lanes 2, 3, 4, 10, 11, 14) or antibodies (lanes 5, 6, 9, 15, 16) indicated. n.im., Nonimmune serum. The quantity of GABP proteins was five times as much for lane 12 as for lanes 13–16. Arrows a–d, specific shifts; n.s., nonspecific; F, free probe. Lanes 1–7, 8–11, and 12–16 correspond to representative independent experiments.

Oligo D, which corresponds to the distal cluster, produced with FTO2B extracts a specific shift that was inhibited by oligo Sp1 and by oligo Dmut*ets* but was not inhibited by oligo DmutSp1 or oligo *ets*, and it bound recombinant Sp1. The affinity of oligo D for Sp1 was much lower than that of oligo C (data not shown). Thus, in FTO2B extracts, the distal and proximal *ets*/Sp1 clusters of the promoter bind Sp1, whereas the central cluster binds *ets*-related proteins. This result is consistent (28–30) with our finding (6) that FTO2B protein binding to this motif is prevented by *in vitro* methylation of the GG core on the sense strand and of an adenosine located 4 bp downstream on the antisense strand.

The association of Sp1 and *ets* motifs in the F promoter was reminiscent of that found in the long terminal repeat of the human T-cell leukemia virus 1. There, Sp1 stimulates DNA binding of, and transactivation by, Ets1 (18). We found by gel shift that oligo B could bind purified Ets1, but this association was not modified by purified Sp1 (data not shown). Moreover, the central *ets* motif of the F promoter (present in oligo B) was not involved in a cooperation with the neighboring Sp1 motifs because its presence stimulated the activity of the *Hph* I–*Taq* I promoter fragment (Fig. 3, construct i) to the same extent when it was associated with these Sp1 motifs (construct h versus e) as when it was not so associated (construct i versus j).

To identify the *ets*-related proteins present in FTO2B cells, gel shifts of oligo B were performed with FTO2B extracts and antibodies that recognize Ets1 and Ets2. These antibodies did not prevent or retard the complexes seen with oligo B (Fig. 4, lane 9), excluding a role of Ets1 or Ets2 in these complexes. FTO2B proteins were therefore separated by SDS/PAGE, and fractions were renatured to determine their ability to bind oligo B. A fraction containing proteins with $M_r$ of 45,000–50,000 produced shifts that comigrated with complexes a, b, and c (data not shown), suggesting the involvement of an oligomeric protein with subunits present in the same fraction. GABP was the only member of the *ets* family known to meet these criteria (30). This heteromeric rat transcription factor, which is the equivalent of human transcription factor EF-1A (31), binds to the immediate early gene of herpes simplex virus and to the adenovirus E1A and E4 and polyomavirus enhancer regions. The 41.3-kDa $\beta$ subunit interacts through ankyrin repeats with the *ets*-like 51.3-kDa $\alpha$ subunit and increases the affinity of GABP$\alpha$ for DNA (30). Consistent with our hypothesis, anti-GABP$\alpha$ antibodies inhibited formation of three (a, b, and c) of the four complexes between oligo B and the FTO2B nuclear proteins (Fig. 4, lane 5). Anti-GABP$\beta$ antibodies inhibited complexes a and b but did not inhibit complex c (lane 6), suggesting that complex c represented binding of the GABP$\alpha$ monomer to oligo B and that complexes a and b were due to binding of GABP$\alpha/\beta$ heteromers. This hypothesis was confirmed with recombinant GABP$\alpha$ and -$\beta$, which produced shifts that comigrated with complexes a, b, and c (lane 12). These shifts were prevented by oligo B and by anti-GABP$\alpha$ antibodies, whereas only the two upper shifts were prevented by anti-GABP$\beta$ antibodies (lanes 13–16).

The binding specificity of the *ets* family members depends on the DNA sequences flanking the *ets* motif (26, 32) and on their protein partner (33). Thus, if GABP is the FTO2B factor that binds to the F promoter, the relative affinity of the FTO2B proteins for the three *ets*/Sp1 clusters of the F promoter should be the same as that of GABP. This was the case, as oligos D and C did not prevent the complexes obtained with oligo *ets*, whereas oligo B did prevent these complexes (Fig. 5). Therefore, the main complexes (a, b, and c) formed between FTO2B proteins and the central motif of the F promoter are contributed by GABP. This transcription factor and Sp1 are considered to be ubiquitous. However, the concentration and phosphorylation-dependent activity of

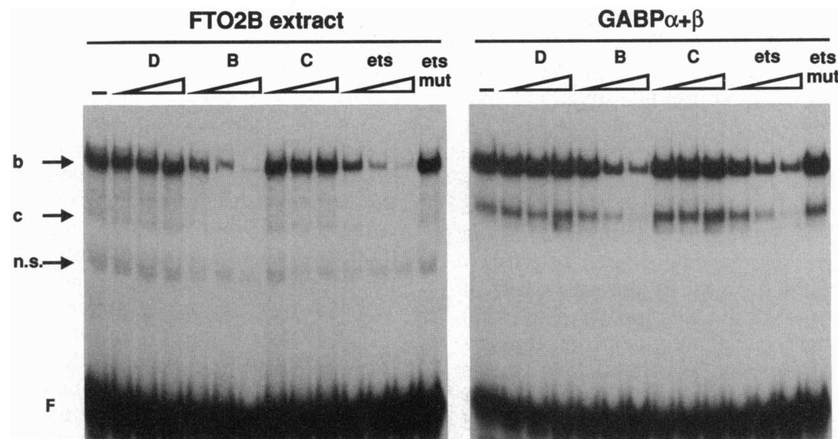


FIG. 5. Relative affinity of F promoter fragments for the FTO2B cell or GABP proteins, evaluated by gel shift of the *ets* probe. Binding reactions contained increased concentrations (10-, 40-, and 100-fold molar excess), indicated by the triangles, of competing oligos D, B, or C (Fig. 1*B*) or *ets*, or a 100-fold molar excess of oligo *ets*mut. Arrows b and c, specific shifts; n.s., nonspecific; F, free probe.

Sp1 family members vary with cell type and developmental stage (34). Posttranslational modifications of Sp1 and, possibly, GABP might explain why F-type mRNA expression appears restricted to proliferating cells. Such control could also be exerted by cis-acting sequences located outside the promoter region described here. Moreover, although the four complexes detected between FTO2B proteins and oligo B were ascribed to *ets*-related proteins, only three were typical of GABP. The minor, faster migrating, complex d could also participate in the activity of the promoter. Finally, the region containing the functional *ets* motif described here behaved as an enhancer of the M promoter (6).

In conclusion, we have characterized a third mRNA and its promoter in a gene already known to encode the L and M isozymes of PFK-2/FBPase-2. The organization of the F promoter differs from that of the L-type and M-type promoters, which both contain a TATA box and binding sites for tissue-specific factors and for CCAAT transcription factor/nuclear factor 1 (6, 20). The F-type mRNA occurs in proliferating tissues such as preterm placenta, fetal liver and muscle, and established lines of mammalian cells. Our data also suggest that its synthesis is controlled by GABP and, perhaps, other *ets*-related transcription factors, and by Sp1. To our knowledge, only viral genes have been described as GABP targets. Several human tumors are associated with abnormal expression of *ets* (35). Identification of cellular genes for which transcription is controlled by *ets* oncogene products is relevant for our understanding of the malignant phenotype.

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1. Weinhouse, S. (1976) *Z. Krebsforsch.* **87,** 115–126.
2. Hue, L. & Rousseau, G. G. (1993) *Adv. Enzyme Regul.* **33,** 97–110.
3. Rousseau, G. G. & Hue, L. (1993) *Prog. Nucleic Acid Res. Mol. Biol.* **45,** 99–127.
4. Darville, M. I., Crepin, K. M., Hue, L. & Rousseau, G. G. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 6543–6547.
5. Crepin, K. M., Darville, M. I., Hue, L. & Rousseau, G. G. (1989) *Eur. J. Biochem.* **183,** 433–440.
6. Darville, M. I., Antoine, I. V. & Rousseau, G. G. (1992) *Nucleic Acids Res.* **20,** 3575–3583.
7. Loiseau, A. M., Rider, M. H., Foret, D., Rousseau, G. G. & Hue, L. (1988) *Eur. J. Biochem.* **175,** 27–32.
8. Cifuentes, M. E., Espinet, C., Lange, A. J., Pilkis, S. J. & Hod, Y. (1991) *J. Biol. Chem.* **266,** 1557–1563.
9. Auffray, C. & Rougeon, F. (1980) *Eur. J. Biochem.* **107,** 303–314.
10. Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 1408–1412.
11. Marchuk, D., Drumm, M., Saulino, A. & Collins, F. S. (1990) *Nucleic Acids Res.* **19,** 1154.
12. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY), 2nd Ed.
13. Davis, L. G., Dibner, M. D. & Battey, J. E. (1986) *Basic Methods in Molecular Biology* (Elsevier, Amsterdam), pp. 143–146.
14. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K., eds. (1987) *Current Protocols in Molecular Biology* (Greene and Wiley, New York), pp. 4.7.1–4.7.8.
15. Dignam, J. D., Lebovitz, R. M. & Roeder, R. G. (1983) *Nucleic Acids Res.* **11,** 1475–1489.
16. Schreiber, E., Matthias, P., Müller, M. M. & Schaffner, W. (1988) *EMBO J.* **7,** 4221–4229.
17. Bosselut, R., Duvall, J. F., Gégonne, A., Bailly, M., Hemar, A., Brady, J. & Ghysdael, J. (1990) *EMBO J.* **9,** 3137–3144.
18. Gégonne, A., Bosselut, R., Bailly, R. A. & Ghysdael, J. (1993) *EMBO J.* **12,** 1169–1178.
19. Ghysdael, J., Gégonne, A., Pognonec, P., Dernis, D., Leprince, D. & Stehelin, D. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 1714–1718.
20. Lemaigre, F. P., Durviaux, S. M. & Rousseau, G. G. (1991) *Mol. Cell. Biol.* **11,** 1099–1106.
21. Crepin, K. M., De Cloedt, M., Vertommen, D., Foret, D., Michel, A., Rider, M. H., Rousseau, G. G. & Hue, L. (1992) *J. Biol. Chem.* **267,** 21698–21704.
22. Luo, X., Park, K., Lopez-Casillas, F. & Kim, K.-H. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 4042–4046.
23. Faisst, S. & Meyer, S. (1992) *Nucleic Acids Res.* **20,** 3–26.
24. Smale, S. T. & Baltimore, D. (1989) *Cell* **57,** 103–113.
25. Woods, D. B., Ghysdael, J. & Owen, M. J. (1992) *Nucleic Acids Res.* **20,** 699–704.
26. Nye, J. A., Petersen, J. M., Gunther, C. V., Jonsen, M. D. & Graves, B. J. (1992) *Genes Dev.* **6,** 975–990.
27. Seth, A., Ascione, R., Fischer, R. J., Mavrothalassitis, G. J., Bhat, N. K. & Papas, T. S. (1992) *Cell Growth Differ.* **3,** 327–334.
28. Gunther, C. V., Nye, J. A., Bryner, R. S. & Graves, B. J. (1990) *Genes Dev.* **4,** 667–679.
29. Gitlin, S. D., Bosselut, R., Gégonne, A., Ghysdael, J. & Brady, J. N. (1991) *J. Virol.* **65,** 5513–5523.
30. Thompson, C. C., Brown, T. A. & McKnight, S. L. (1991) *Science* **253,** 762–768.
31. Bolwig, G. M., Bruder, J. T. & Hearing, P. (1992) *Nucleic Acids Res.* **20,** 6555–6564.
32. Wasylyk, C., Kerckaert, J. P. & Wasylyk, B. (1992) *Genes Dev.* **6,** 965–974.
33. Brown, T. A. & McKnight, S. L. (1992) *Genes Dev.* **6,** 2502–2512.
34. Saffer, J. D., Jackson, S. P. & Annarella, M. B. (1991) *Mol. Cell. Biol.* **11,** 2189–2199.
35. Delattre, O., Zucman, J., Plougastel, B., Desmaze, C., Melot, T., Peter, M., Kovar, H., Joubert, I., de Jong, P., Rouleau, G., Aurias, A. & Thomas, G. (1992) *Nature (London)* **359,** 162–165.