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The α_1-Fetoprotein Locus Is Activated by a Nuclear Receptor of the *Drosophila* FTZ-F1 Family

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The α_1-fetoprotein (AFP) gene is located between the albumin and α-albumin genes and is activated by transcription factor FTF (fetoprotein transcription factor), presumed to transduce early developmental signals to the albumin gene cluster. We have identified FTF as an orphan nuclear receptor of the *Drosophila* FTZ-F1 family. FTF recognizes the DNA sequence 5′-TCAAGGTCA-3′, the canonical recognition motif for FTZ-F1 receptors. cDNA sequence homologies indicate that rat FTF is the ortholog of mouse LRH-1 and *Xenopus* xFF1rA. Rodent FTF is encoded by a single-copy gene, related to the gene encoding steroidogenic factor 1 (SF-1). The 5.2-kb FTF transcript is translated from several in-frame initiator codons into FTF isoforms (54 to 64 kDa) which appear to bind DNA as monomers, with no need for a specific ligand, similar K_ds ($\simeq 3 \times 10^{-10}$ M), and similar transcriptional effects. FTF activates the AFP promoter without the use of an amino-terminal activation domain; carboxy-terminus-truncated FTF exerts strong dominant negative effects. In the AFP promoter, FTF recruits an accessory *trans*-activator which imparts glucocorticoid reactivity upon the AFP gene. FTF binding sites are found in the promoters of other liver-expressed genes, some encoding liver transcription factors; FTF, liver α_1-antitrypsin promoter factor LFB2, and HNF-3β promoter factor UF2-H3β are probably the same factor. FTF is also abundantly expressed in the pancreas and may exert differentiation functions in endodermal sublineages, similar to SF-1 in steroidogenic tissues. HepG2 hepatoma cells seem to express a mutated form of FTF.

The albumin gene family consists of four known genes expressed by the liver and encoding serum albumin, α_1-fetoprotein (AFP), α-albumin, and the vitamin D-binding protein. The four genes evolved from a common ancestor and remained tightly linked in the genome (47), suggesting a selective regulatory advantage for their communal hepatocytic expression. However, the tandem loci are subject to strong differential expression during liver development, and this provides a powerful approach to molecular controls of liver cell differentiation. Much work on the multigene array has focused on AFP for its unique responses to early developmental growth and hormonal signals. AFP is triggered first, when gut endodermal cells become committed to hepatic lineages, then AFP is strongly induced throughout fetal liver growth, along with its 5′ neighbor albumin, followed by near-complete deactivation of AFP in the perinatal period, as the downstream α-albumin and vitamin D-binding protein loci are activated (6, 7, 10, 16, 47). Also, glucocorticoid hormones can abruptly arrest liver AFP gene transcription (5, 21), while neoplastic transformation of hepatocytes often reactivates AFP. Thus, the albumin multigene control and AFP modulations promise insights not only into the archetype liver chromatin domain but also into how eukaryotic genes are up or down regulated by carcinoembryonic and glucocorticoid signals.

One simple postulate regarding AFP is that it could be specifically regulated by a differentiation factor coupling AFP activation with fetal nutritive functions of early endodermal cells. Molecular biological data have supported the existence of such a specific activator. Of the promoter and enhancer elements controlling albumin-related loci, one *cis*-activatory component was found essential and unique to the AFP locus, and furthermore it mapped to a discrete AFP promoter segment repressed by dexamethasone (see Fig. 1). Mutation-transfection and DNA-protein binding analyses circumscribed a conserved 12-bp *cis*-activatory element at −160 bp from the AFP transcription initiation site and strongly bound by a liver-enriched fetoprotein transcription factor (FTF) (7). Quite notably, the 12-bp sequence displayed a pseudopalindromic arrangement of two near-canonical nuclear receptor recognition motifs AGGTCA: this hinted that a receptor-like factor might transduce early developmental signals to unfold the AFP multigene domain.

Here, we have further characterized FTF from rat liver. Rat FTF (rFTF) is an orphan nuclear receptor, the ortholog of mouse liver receptor homolog 1 (mLRH-1) (61), human PHR-1 (4), and frog xFF1rA (18), all related to the *Drosophila fushi tarazu* F1 (FTZ-F1) (36) receptor. The activation of AFP and structure and distribution of FTF suggest new roles for FTZ-F1 receptors in mammalian development and endodermal cell differentiation, with other potential gene targets and important selective cross-talk with the glucocorticoid receptor (GR).

MATERIALS AND METHODS

cDNA cloning and expression vectors. A rat liver cDNA library in phage vector ZAPII (catalog no. 936513; Stratagene) was screened by standard plaque hybridization at 55°C (54) with a mLRH-1 cDNA fragment spanning 130 nucleotides in the predicted DNA-binding region (61) (nucleotide positions 461 to 590 [see Fig. 3A]). Synthetic oligonucleotides spanning the upper DNA strand from nucleotides 461 to 537 and the lower DNA strand from nucleotides 515 to 590 were annealed and ^{32}P labeled by filling the partial duplex with the Klenow

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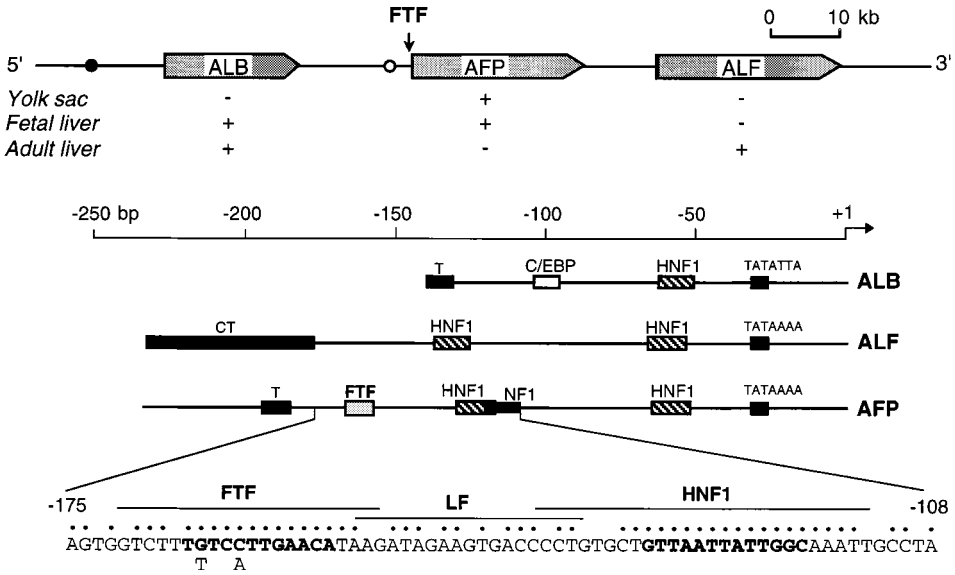

FIG. 1. Functional structure of the rat albumin (ALB), AFP, and α-albumin (ALF) genes. The diagram presents the three genes transcriptionally oriented on chromosome 14, with their relative expression (expressed [+] or not expressed [−]) in rat visceral yolk sac and hepatocytes; a fetal enhancer (7) (open circle) and an adult enhancer (extrapolated from mouse studies) (52) (closed circle) are shown. Promoter structures are compared with their TATA elements and 5′-flanking homopolymers T/A or CT/GA, common binding sites for HNF-1, a C/EBP-activatory site in albumin, and AFP-specific FTF-activatory and NF1-repressive sites. The upstream AFP promoter is shown with its FTF and HNF-1 recognition sequences (7, 69) in bold type; dots indicate nucleotides conserved among species, and overlined sequences mark DNase I footprints from FTF, HNF-1, and FTF accessory factor LF in liver nuclear extracts; mutation GT→CA inactivates the AFP promoter in hepatocyte transfection assays (22).

polymerase. The probe retrieved four different recombinant phages from 10^6 plaques. The inserts were sequenced on both strands by using the T7 dideoxy sequencing system (Pharmacia), and the longest insert was subcloned in pRcRSV (Invitrogen) (construct pfΔ3 [see Fig. 6B]). Vector pf1 contains the mLRH-1 cDNA in plasmid pSG5, with an *Eco*RI site and an optimized translation initiation sequence preceding the first in-frame AUG codon (31) (see Fig. 6B). Plasmid pCIf1 contains the pf1 cDNA insert transferred into vector pCI (Promega). Vector pf4 is the N-terminus-truncated derivative (see Fig. 6B) of pf1, obtained by substituting the *Eco*RI-*Afl*II fragment of pfΔ3 into pf1. Vectors pfΔ1 and pfΔ2 are functionally C-terminus-truncated mutants (see Fig. 6B) of pCIf1, obtained by introducing translational stop codons at amino acid G-543 or R-526. All constructs were verified by sequencing. Expression vectors RSV-HNF-1α and pRShGRα encode rat hepatocyte nuclear factor 1α and human GRs (7). Reporter chloramphenicol acetyltransferase (CAT) vectors are documented below.

DNA-protein binding assays. Total nuclear protein extracts were prepared from fresh tissues as described previously (7). The isolation of nuclei from cultured cells was performed by a previously published method (14) with minor adjustments: nuclei were then processed in the same way whole tissues were (7). Electromobility shift assays were performed as previously detailed (7) with 0.2 ng of ^{32}P-labeled oligonucleotide probe, 2 to 5 μg of nuclear proteins or 1 μl of reticulocyte transcription-translation lysate (TNT system; Promega), and 10- or 20-fold molar excess of competitor oligonucleotides (generally blunt or with *Eco*RI overhangs). Protein extracts giving no specific reaction with the FTF probe 5′-TGTTCAAGGACA-3′ were validated by specific band shifts with a probe for (ubiquitous) NF1. Saturation binding analysis was performed with a constant amount of protein (rat liver, 25 μg; HepG2, 5 μg; reticulocyte lysate, 2.5 μl) and concentrations of FTF probe increasing from 13.9 pM to 2.14 nM: protein-bound and free probes were recovered from dried electrophoretic gels and counted, and dissociation constants were derived by Scatchard plot transformation. For supershift experiments, rabbits were immunized with the synthetic peptide (one-letter code) CLTSAIQNIHSASKGL (rFTF positions 142 to 156 in Fig. 3B, plus an amino-terminal cysteine), purified by high-pressure liquid chromatography and coupled to keyhole limpet hemocyanin (34). Preimmune or immune serum (1 μl) was incubated with nuclear extract for 2 h at 4°C, followed by 30 min of incubation at 0°C with the probe and electrophoresis. For DNA-FTF methylation interference assay, oligonucleotides spanning the upper or lower strand of AFP promoter segment from nucleotides −151 to −172 (Fig. 1) were labeled with [γ-^{32}P]ATP and the T4 polynucleotide kinase (Gibco-BRL), then partially methylated at guanine and adenine residues with dimethyl sulfate (54), and annealed with cold complementary oligonucleotide. The double-strand probes (3 ng) were used in electromobility shift assays with 5 μg of rat liver nuclear extract; protein-bound and free probes were digested with piperidine and analyzed on 15% polyacrylamide–6 M urea sequencing gels (54).

Transfection assays. Stable transfection assays were conducted in a clonal variant (7777.6) of 7777 rat hepatoma Morris cells, and transient expression assays were conducted in human HepG2 or HeLa cells, as detailed previously (7, 22). AFP-CAT vectors 5200 and mUP-TK40 have been described elsewhere (7). New constructs were derived from AFP-CAT plasmid 4400, obtained by inserting the *Bam*HI AFP-CAT fragment of vector 7000 (22) into the *Bam*HI site of pBluescript SK$^+$ (Stratagene). To facilitate further manipulations, unique restriction sites were introduced in plasmid 4400 at AFP positions −89 (*Mlu*I) and −138 (*Nar*I) (vector m4) (these base changes were neutral in transfection assays). Mutations were then targeted in vector m4 at FTF-footprinted nucleotides −155/−156/−157 (vector m5), −158/−159/−160 (m6), −161/−162/−163 (m7), −164/−165/−166 (m8), and −167/−168/−169 (m9) by PCRs. Two other AFP-CAT constructs were derived from previously described pSV0-AFP-cat vectors (22). Vectors E4-202 and E4-158 contain AFP enhancer segment from nucleotides −4000 to −2124 fused to AFP promoter segments from nucleotides −202 to +5 or −158 to +5 linked to CAT in pSV0. FTF-footprinted AFP promoter segment from nucleotides −173 to −151 was also tested in single or multiple copies (as a synthetic oligonucleotide with *Eco*RI overhangs) upstream of the 105-bp thymidine kinase (TK) promoter (containing SP1 and NF1 binding sites) or of the minimal 40-bp TK promoter in pTK-CAT (29); FTF-LF and FTF–LF–HNF-1 promoter segments were similarly introduced in single copies in front of TK105; the FTF oligonucleotide (with *Bgl*II overhangs) was further inserted as a multimer upstream of the multi-SP1-site simian virus 40 (SV40) promoter in $pA_{10}cat_2$ (32).

RESULTS

FTF DNA-binding site. Our previous analysis of the FTF binding element in the rat AFP promoter used crude liver nuclear extracts to circumscribe a 22-bp DNase I-footprinted segment between −151 and −172 nucleotides from the transcription initiation site (Fig. 1). (When used as a probe in gel shift assays, the 22-bp footprinted segment generated [depending on the species and cell lines] one to four specific bands, in close proximity and with similar affinity, thermosensitivity, and reactivity to binding site mutations (7); we thus provisionally assigned the heterogeneous pattern to isoforms of a single factor, FTF). To better map the FTF recognition site, G-residue contact points were analyzed by methylation interference assay. The results (Fig. 2A) indicated that G contacts were all contained in a 12-bp DNA sequence centering the footprint with two nuclear receptor-binding hexameric motifs:

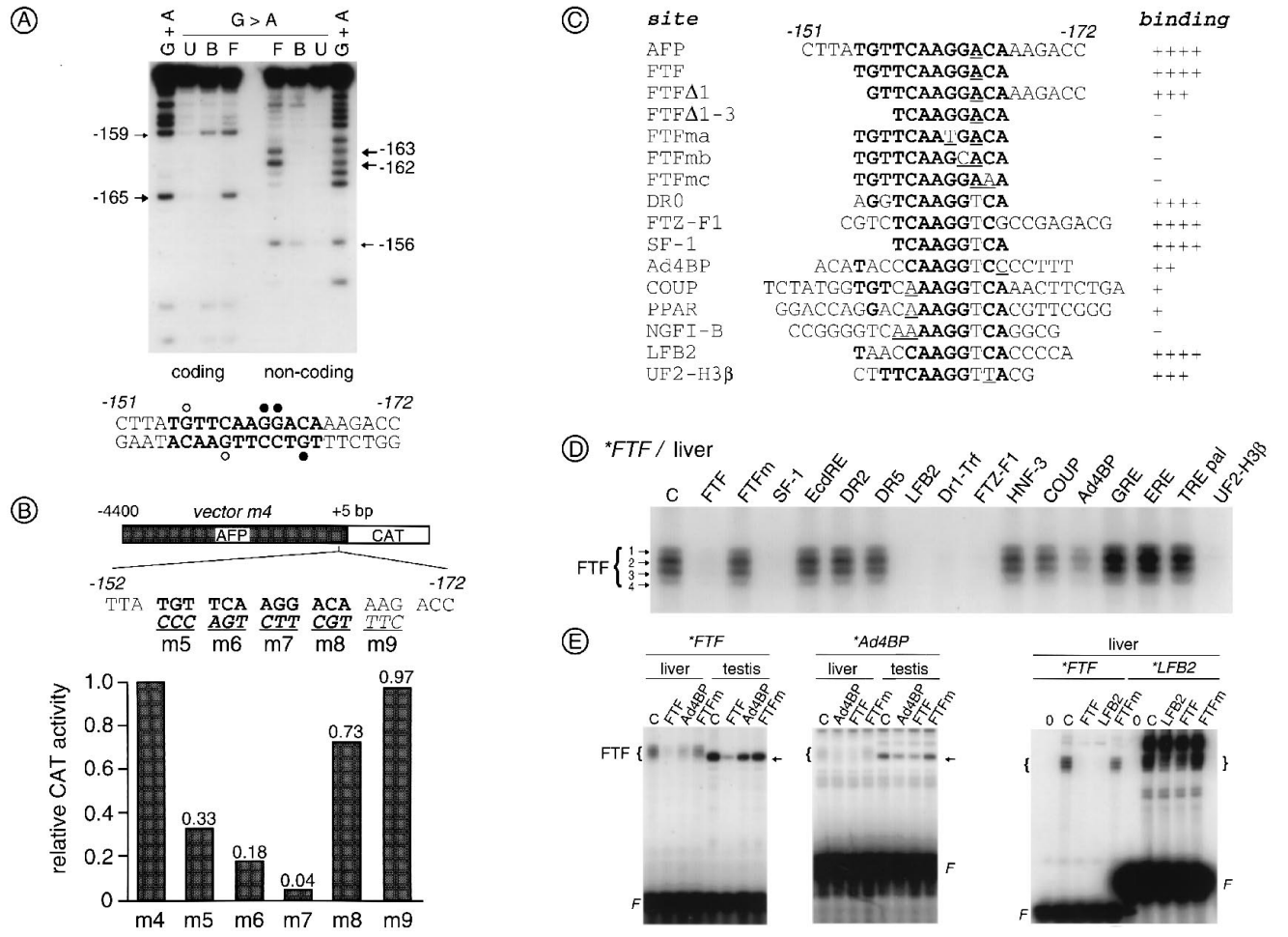

FIG. 2. Characterization of the FTF DNA-recognition sequence. (A) Methylation interference analysis using liver nuclear extract and rat AFP promoter segment from nucleotides −151 to −172. The autoradiogram shows chemical cleavage reactions at G and A residues (G>A) in FTF-bound (B) or unbound (F) DNA probes radiolabeled on the coding or noncoding strand (U is unmethylated protein-free DNA; G+A are reference sequencing reactions). Arrows highlight DNA fragments enriched in free probe (lanes F), summarized at the bottom as strong (●) or weak (○) G-contact points. (B) Mutational analysis of the FTF binding site by stable transfection in 7777.6 rat hepatoma cells. CAT activities relative to parental AFP enhancer-promoter vector m4 were averaged from three highly reproducible assays. (C) FTF-binding activities tested by competitive electromobility shift assays (liver extract and AFP probe from nucleotides −151 to −172). Nucleotides in bold type match the AFP FTF sequence, and those underlined do not match the sequence PyCAAGGPyCPu. (D) Autoradiogram from an electromobility shift assay using the radiolabeled probe TGTTCAAGGACA (*FTF) and 2.5 μg of nuclear proteins from rat liver, without (lane C) or with 10-fold excess of the indicated competitors. Sequences FTF, SF-1, LFB2, FTZ-F1, COUP, Ad4BP, and UF2-H3β are shown in panel C. FTFm is TGTTCAATGAAA. Ecdysone response element EcdRE is AGGTCATTGACCC. DR2 and DR5 are direct AGGTCA repeats separated by AA and AATTA, respectively. Transferrin promoter element Dr1-Trf is GGGCT CAAGGTCAAAG (the related transferrin Pr1 element [9] did not compete). HNF-3 is the 25-bp HNF-3 binding site in the transthyretin promoter (13). GRE, ERE, and TREpal are palindromic consensus sequences for glucocorticoid, estrogen, and thyroid hormone receptors, respectively. (E) Electromobility shifts with FTF, Ad4BP, and LFB2 probes (sequences as in panel C) and nuclear extracts from adult rat liver or testis. Lanes 0 contain no extract, lanes C contain no competitor, and other lanes contain 20-fold molar excess of cold competitor (sequences given in panels C and D); braces and arrows mark specific band shifts. *F*, free probe.

161-AGGACA-166, close to the estradiol receptor (ER) motif AGGTCA, and 160-TGAACA-155, close to the GR motif AGAACA (3). The three G's in the ER-type hexamer had strong contacts with FTF, while the two G's in the GR-type hexamer made weaker contacts, indicating that the lower-strand hexamer was the preponderant FTF-recognition site. Point mutations were then targeted to the 12-bp sequence in the natural 4.4-kb AFP enhancer-promoter context and tested by stable transfection assays in 7777.6 rat hepatoma cells. The results (Fig. 2B) confirmed that each of the four G-contacted base triplets contributed to AFP promoter activity; the most dramatic effect (>95% deactivation) was obtained by altering the double-G contact in the ER-type FTF-binding hexamer (i.e., the AGG triplet). These data provided a functional relationship in vivo with the FTF-DNA binding experiments in vitro, confirming a two-hexameric-receptor-like recognition site, with a critical ER hemihexameric submotif AGG.

We next used electromobility shift assays to refine the FTF-DNA binding requirements in relation to other nuclear receptor-binding sites (Fig. 2C, D, and E). As expected, the FTF binding affinity of a 12-bp FTF probe was similar to our initial 22-mer probe (spanning the FTF footprint) and achieved complete band shift displacement with ≃10-fold molar excess of cold oligonucleotide competitor (the 12-bp FTF probe also resolved nuclear extracts into much cleaner patterns of tightly split bands). As anticipated, mutations at strong G-contact points (FTFma, FTFmb, and FTFmc in Fig. 2C) or truncation of a triplet (FTFΔ1-3) or even of a single base (FTFΔ1) abolished or significantly reduced binding, confirming the stringent requirement for a two-hexameric sequence in the AFP pro-

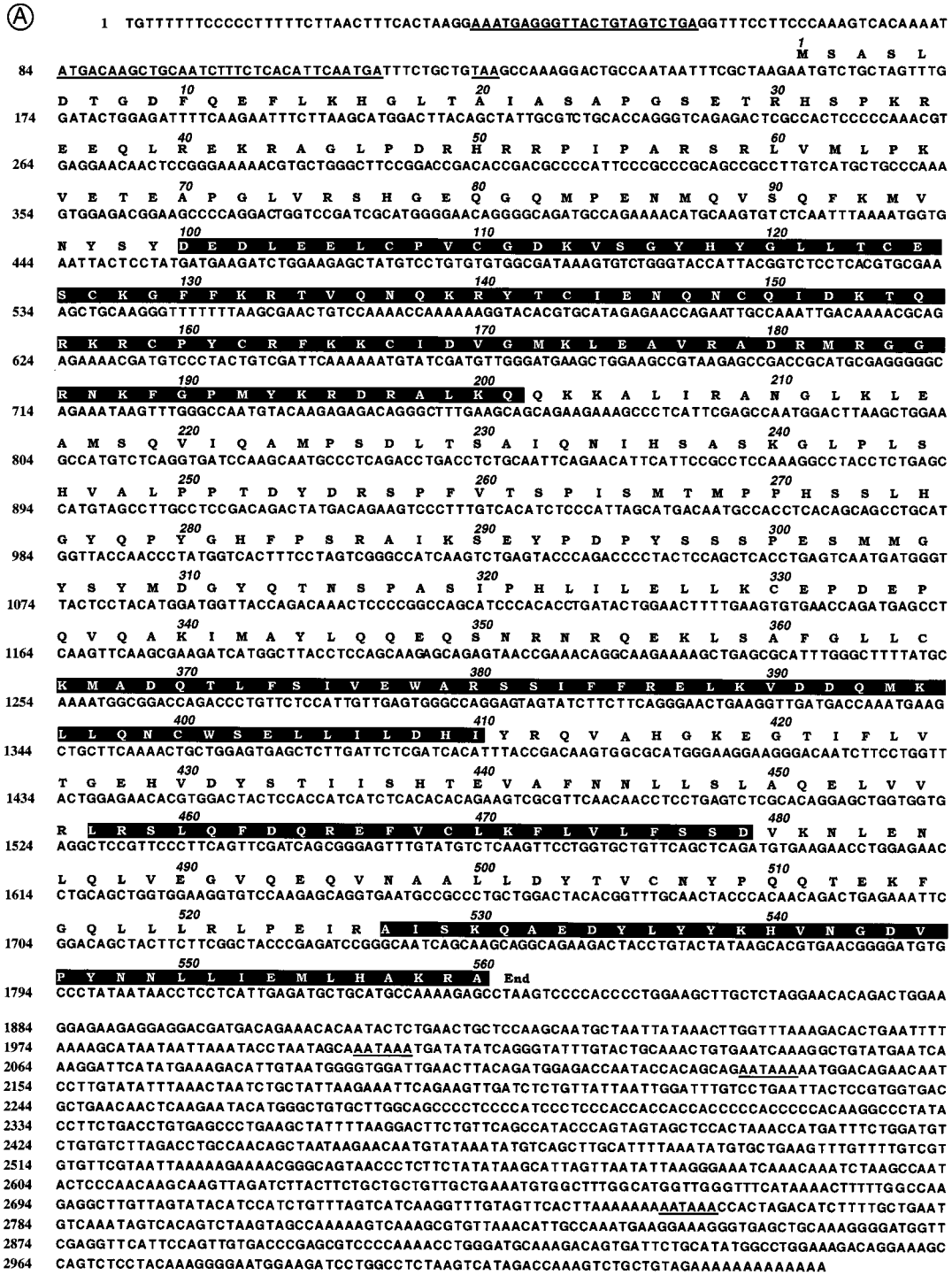

FIG. 3. (A) Complementary DNA sequence and predicted amino acid sequence of mLRH-1 (61). A mouse liver cDNA library was screened with a degenerate oligonucleotide probe based upon a conserved segment of the DBD of nuclear hormone receptors, as previously described (28); one mLRH-1 clone (clone 74 in reference 28) containing a 1.4-kb mLRH-1 cDNA fragment was used for further screening. Double-stranded DNA sequencing was performed on nested deletions with exonuclease III (Stratagene) and modified T7 polymerase (Sequenase; U.S. Biochemicals). Nucleotide positions are shown on the left, predicted amino acid sequence and numbering are above the nucleotide sequence. Two short open reading frames preceding the large open reading frame, an upstream in-frame termination codon, and three potential polyadenylation signals are underlined. Stretches of white amino acids on black background are regions of receptor homologies diagrammed in Fig. 6B. (B) Amino acid alignment of rFTF with other orphan and steroid receptors: mouse LRH-1 (61), *Xenopus* FF1rA (xFF1rA) (18), mouse SF-1 (mSF-1) (33), *Drosophila* FTZ-F1 (dFTZ-F1) (37), *Drosophila* DHR39 (dDHR39) (2, 49), rat nerve growth factor-induced protein B (rNGFI-B) (43), rat chicken ovalbumin upstream promoter transcription factor (rCOUP) (12), rat GR (42), and rat ER (30). Sequences were aligned from the predicted FTF DBD. Numbers on the left mark the position of the first aligned residue relative to the N-terminal methionine. White residues on black background match the FTF sequence. Zinc finger motifs I and II are shown. Arrowheads point to the Zn-coordinated cysteines. Introns are mapped from GR and ER genes. Arrows mark a heptad repeat in FTF, and asterisks locate the FTF peptide used to raise antibodies.

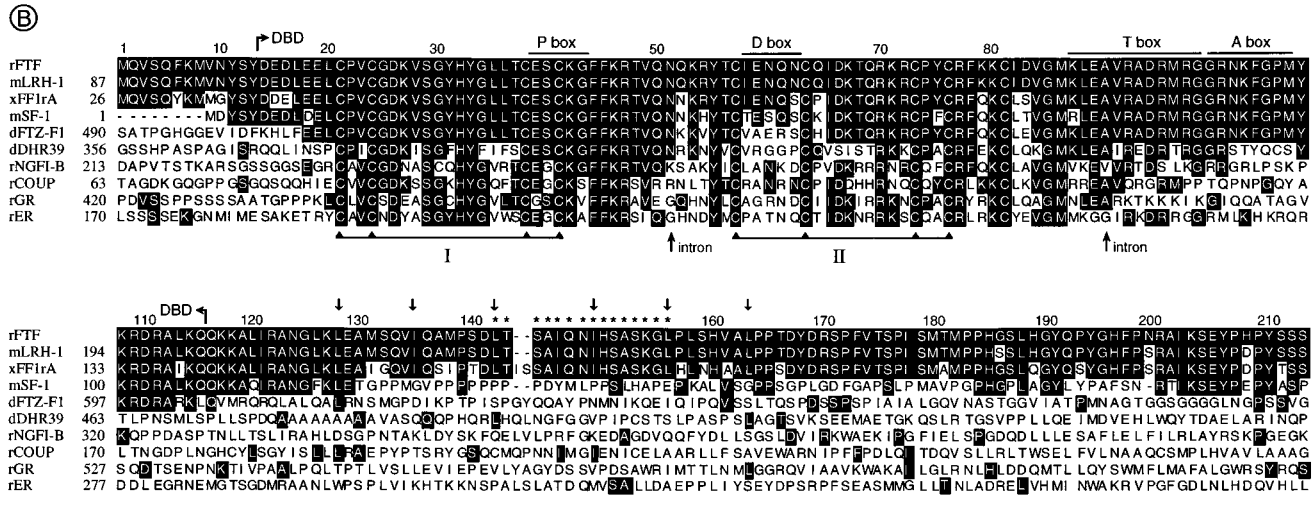

FIG. 3—*Continued.*

moter context. We then tested canonical two-hexameric receptor binding elements, including all documented palindromic steroid and thyroid receptor binding sites, and retinoid receptor-type motifs AGGTCA in the form of direct repeats with a 1- to 5-nt spacer (DR1 to DR5). None of these elements had FTF binding capacity approaching that of the natural AFP promoter sequence (results are partly illustrated in Fig. 2D). We found however that a direct repeat with no spacer (DR0) bound FTF as efficiently as the AFP promoter (Fig. 2C). This was a defining point because DR0 encompassed the consensus nonameric sequence PyCAAGGPyCPu which binds orphan receptors of the FTZ-F1 or SF-1 (steroidogenic factor 1) classes (55); furthermore the three strong G contacts in the AFP FTF-binding site matched the same contacts in the FTZ-F1 core binding hexamer of the *fushi tarazu* Zebra element (36). Further assays showed that the natural FTZ-F1 recognition sequence (36) or the minimal SF-1 binding site TCAAGGTCA (45) could bind FTF as efficiently as the natural AFP promoter motif (Fig. 2C and D). This indicated that the extra 5′ TGT triplet in the 12-bp AFP motif could compensate for the T-to-A substitution in its core hexamer (TCAAGGACA). That FTF might be an orphan receptor of the SF-1 or FTZ-F1 subclass was further tested by changing the 6-bp context upstream from the consensus core motif AG GTCA; orphan receptors of different subclasses detect slight differences in the proximal or distal upstream triplets, discriminated for by short specific segments of the receptor DNA-binding domain (DBD) (19, 64, 68). Results with natural orphan receptor COUP and PPAR sequences (Fig. 2C and D) showed that changing a single base in the FTZ-F1 proximal triplet (CCA to CAA) reduced substantially the capacity to bind FTF. A further 1-bp change in the proximal triplet (CAA to AAA), forming an optimal binding site for orphan receptor NGFI-B (68), destroyed all FTF binding (Fig. 2C): NGFI-B and SF-1 differ by only a few amino acids to discriminate the proximal triplet (68), and this strongly indicated that FTF and SF-1 should share closely related DBDs.

The FTF recognition sequence predicted that the AFP promoter should be recognized by SF-1 in steroidogenic cells and that SF-1-binding elements from steroidogenic genes might recognize FTF in liver extracts. Indeed, the FTF probe detected an abundant factor in gonadal tissues and placenta (testis [Fig. 2E] and placenta and ovary [see Fig. 8O and P]), sensitive to the same binding site mutations that eliminate FTF binding. The FTF sequence in fact was a better binding site than the natural bovine SF-1 recognition element Ad4BP (45) (presumably because of the Pu→Py substitution in the core hexamer [AGGTCC] [Fig. 2C]): while SF-1 binding was easily detected with a natural Ad4BP probe (Fig. 2E, testis), only faint specific bands were detected in liver extract (Fig. 2E), which probably explains why rFTF went previously unnoticed in SF-1 studies (45). The FTF motif also allowed a search for other FTF-regulated promoters. The transferrin and α_1-antitrypsin genes, coexpressed with AFP in the liver or yolk sac (20) do contain consensus FTF-binding sites in their promoters. One corresponds to element Dr1 in the distal transferrin promoter (9), and the other corresponds to element LFB2 (liver factor B2) in the proximal α_1-antitrypsin promoter (44). An LFB2 probe generates the typical FTF band shift pattern with the same sensitivity to FTF site mutations, strongly equating factor LFB2 with FTF (Fig. 2E) (44). Potential FTF-activated sites were also found in liver-expressed genes encoding transcription factors. Among canonical or near-canonical sequences, one has been characterized in the proximal HNF-3β gene promoter (51). The HNF-3β DNA segment from nucleotides −51 to −24 encompasses a one-mismatch FTF motif: it strongly competes for FTF binding in band shift assays (Fig. 2C and D), and a HNF-3β probe from nucleotides −51 to −24 displays the typical liver FTF bands dependent on FTF G-contact points (factor UF2-H3β in reference 51). Thus, liver factors UF2-H3β, LFB2, and FTF appear to be the same factor.

Putative rat FTF cDNA. The FTF DNA-recognition sequence TCAAGGTCA with the specific requirements in the first base triplet indicated that the DBD of FTF should be closely related to those of FTZ-F1 and SF-1. A data bank survey of FTZ-F1-related DBDs retrieved one mammalian sequence, in addition to SF-1, with the proper P-box element to recognize the core hexamer AGGTCA and the proper T and A boxes to discriminate the complementary TCA triplet (41, 65). That sequence belonged to mLRH-1 (Fig. 3A). An mLRH-1 DBD probe was used to screen a rat liver cDNA library, and four different clones were retrieved and sequenced over ≃600 nucleotides (GenBank accession number U47280). The four clones contained identical sequences, suggesting that they came from one locus; and the rat clones were nearly

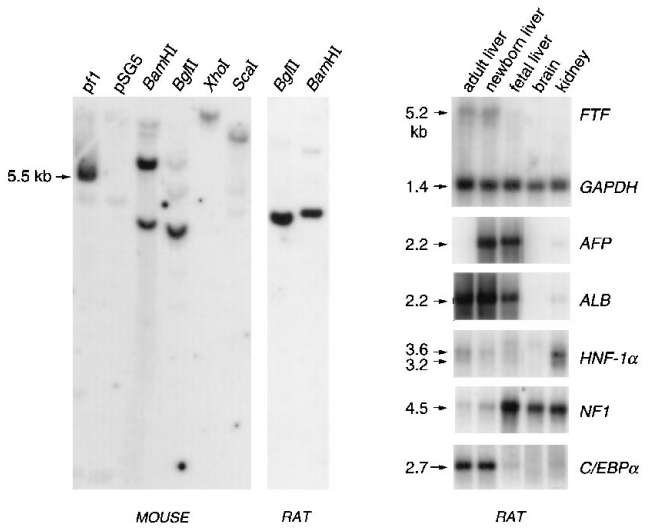

FIG. 4. Southern and Northern analyses with rFTF and mLRH-1 probes. (Left) Southern blots. Fifteen micrograms of genomic DNA (from WW6 mouse or Sprague-Dawley rat) was digested with the indicated enzymes, blotted onto Hybond-N nylon membranes (Amersham), and hybridized at low stringency with mLRH-1 (*MOUSE*) or rFTF (*RAT*) cDNA inserts of vectors pf1 and pfΔ3 shown in Fig. 6B (random ^{32}P-labeled probes using the Amersham Multiprime kit). Lanes pf1 and pSG5 are digestions of mLRH-1 expression plasmid pf1 (releasing the 5.5-kb cDNA insert) and parental vector pSG5. (Right) Northern blots. Tissues were pooled from 10 to 30 Sprague-Dawley rats, total RNA was extracted in guanidium and cesium chloride, poly(A)$^+$ RNA was enriched on oligo(dT)-cellulose, and 3 μg of poly(A)$^+$ RNA was separated in 1.5% agarose-formaldehyde gels, blotted onto nylon membranes, and hybridized (54) with radiolabeled rFTF cDNA or with cDNA probes for rat glyceraldehyde 3-phosphate dehydrogenase (GAPDH), rat AFP, rat albumin (ALB), rat HNF-1α, porcine NF1, or rat C/EBPα. Autoradiographic exposure was 7 times longer for FTF than for HNF-1α, NF1, or C/EBPα. The two HNF-1 mRNAs (3.6 and 3.2 kb) correspond to variants α and β (59).

identical to mLRH-1 and *Xenopus* liver receptor xFF1rA (18), not only in the DBD segment but also in N- and C-terminus-adjacent domains that greatly diverge between receptor subclasses (Fig. 3B). The rat clone (and candidate FTF mRNA) was thus most likely a liver ortholog of orphan receptors mLRH-1 and xFF1rA. Consistent with the FTF binding site, the rat cDNA sequence predicted a zinc finger receptor with the AGGTCA recognition P box ESCKG (41, 65) and the 12 T- and A-box amino acids (19, 64, 68) (positions 90 to 101 in Fig. 3B) needed to discriminate the complementary triplet TCA (compare for instance the T- and A-box segment of rat orphan receptor NGFI-B [Fig. 3B], which fully binds TAAAG GTCA but TCAAGGTCA not at all [68]).

Southern and Northern analyses. Rat genomic DNA cleaved with several restriction enzymes and probed with rFTF cDNA yielded one strongly hybridizing DNA fragment, with one or two occasional minor bands (Fig. 4); similar results were obtained with mouse genomic DNA hybridized with a (longer) mLRH-1 probe, i.e., one or two major fragments with few additional components (Fig. 4). These results indicated that the candidate rFTF mRNA, like mLRH-1 mRNA, was transcribed from a single gene locus (minor signals possibly arising from cross-hybridization with the DBD-related SF-1 gene). One locus was thus coherent with only one rFTF mRNA being represented in four independent liver cDNA clones. The four FTF bands in electromobility shift assay with rat liver nuclear extract, if indeed encoded by that one locus, would then have to be different gene products from alternative transcription units (like SF-1 and ELP) (26, 48) or gene product isoforms derived from alternative posttranscriptional processes. In Northern blots, the rFTF cDNA probe detected a ≃5.2-kb poly(A)$^+$ transcript in adult rat liver and a mLRH-1 probe detected a similar-size transcript in adult mouse liver; no rFTF or mLRH-1 mRNAs were detectable in adult kidney, brain, heart, fat, thymus, skeletal muscle, or testis (Fig. 4 and data not shown). The similar sizes of rat and mouse mRNAs again suggested that rFTF and mLRH-1 were ortholog gene transcripts, and their similar tissue distribution correlating with FTF in band shift assays also supported a translation product relationship. There was no indication of a heterogeneous mRNA to explain FTF variants in electromobility shift assays, i.e., if the rFTF mRNA was the only FTF template, then FTF variants would have to be translational or posttranslational isoforms. The weak hybridization signal (compare for instance with transcription factors HNF-1α and C/EBPα [Fig. 4]) also indicated a very low steady-state level of the putative FTF message.

Supershift assays. To establish a more direct link between the rat liver rFTF cDNA clone and the rat liver FTF proteins, we took advantage of peptide specificities predicted from the cDNA clone. A 15-amino-acid sequence in a region of high diversity among nuclear receptors, adjacent to the DBD (asterisks in Fig. 3B), was used to raise antibodies for electromobility supershift assays. The antibodies recognized all four liver FTF fractions (and not at all SF-1 from testis or placenta) (Fig. 5). This showed that the epitope selected from the cloned cDNA sequence was present in all FTF variants defined by liver band shift assays. As sequence homology predicted for mLRH-1 and xFF1rA (Fig. 3B), rat FTF antibodies supershifted all specific complexes formed with the FTF probe in mouse and frog livers (Fig. 5). Because mLRH-1 cDNA detects only one mRNA and xFF1rA displays only pseudoallele or splice variants (17, 18), the data thus indicate that liver FTF orthologs of vertebrates all originate from a single locus.

FTF translational variants and deletion mutants. Fractionation of rat liver nuclear extracts by molecular sieving indicated that FTF fractions in band shift assays were close molecular size variants of decreasing mass from the slower to the faster retarded bands (Fig. 6A). If derived from a single mRNA, such variants could result from alternative posttranslational processes or they could be different translation products. The mLRH-1 cDNA expression vector (pf1) was used determine if the putative FTF mRNA from mice would generate the hepatic band shift pattern in FTF-negative cells or in a cell-free translation system. Notably, the predicted amino acid sequence of pf1 displayed five in-frame methionine codons preceding the DBD (Fig. 6B), all in favorable base context for translation initiation (31). When expressed in HeLa cells or reticulocyte lysates, vector pf1 released three band shift products migrating

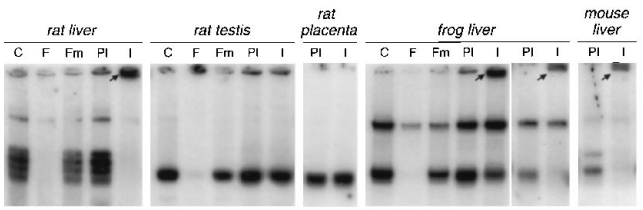

FIG. 5. Supershift assays using antibodies against a specific FTF epitope predicted from cDNA sequences in Fig. 3. Nuclear protein extracts (3 μg) were incubated for 2 h at 0°C with preimmune (PI) or immune (I) serum and then incubated for 30 min at 4°C with the radiolabeled FTF probe (TGTTCAAGGACA); DNA-protein complexes were revealed by electrophoresis and autoradiography. Lanes C, F, and Fm, control reactions with no competitor or with a 20-fold molar excess of cold FTF probe or oligonucleotide FTFm (Fig. 2). Only liver-specific bands were supershifted (arrows).

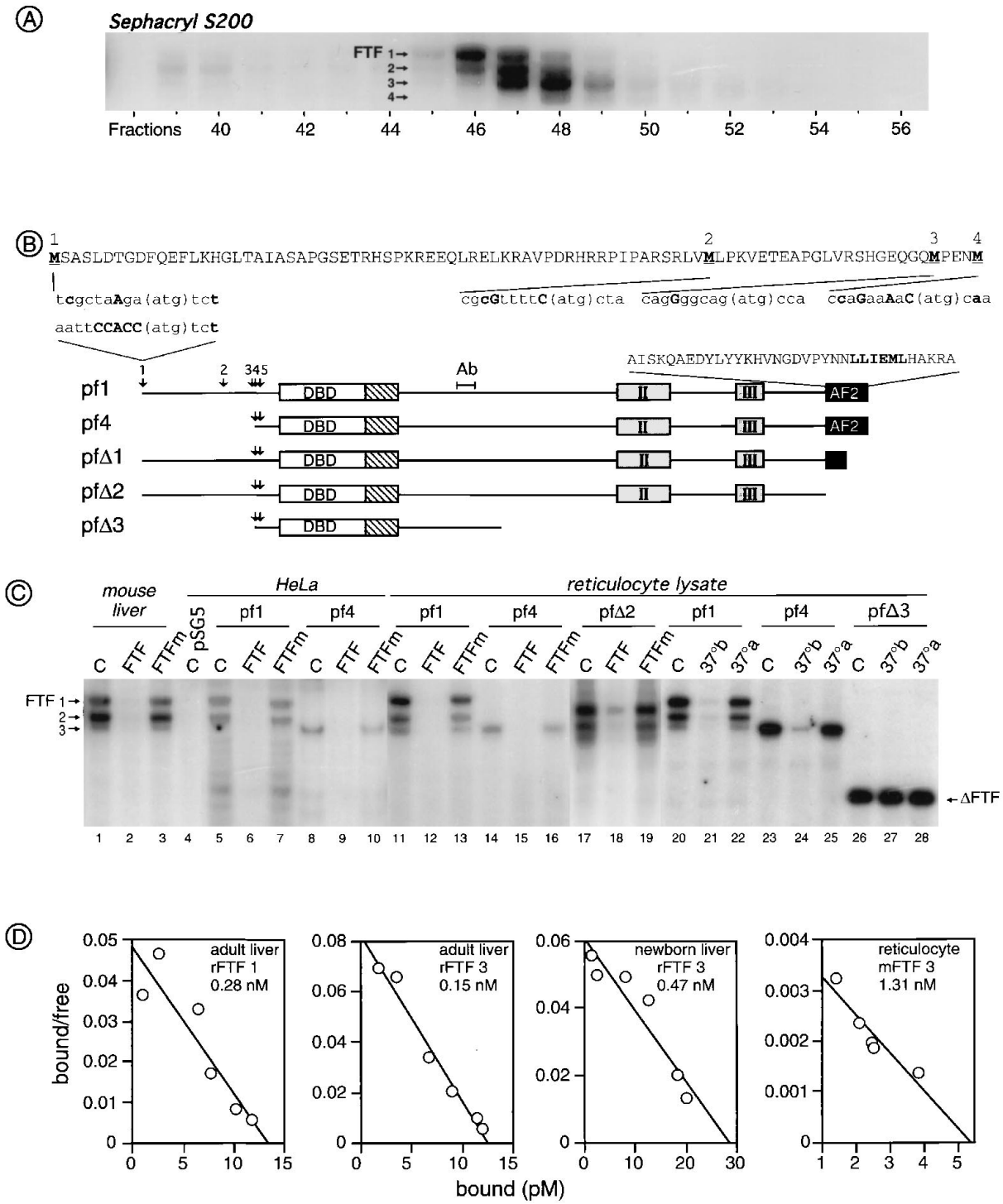

FIG. 6. Characterization of FTF isoforms. (A) Fractionation of native FTF in adult rat liver by chromatography of crude nuclear protein extract on Sephacryl S200; the autoradiogram presents band shift assays of consecutive elution fractions with the FTF probe; the four discrete FTF bands elute like close molecular size variants, precluding monomer-dimer relationships between bands. (B) Predicted proteins from mLRH-1 (pf1, pf4, pfΔ1, and pfΔ2) and rFTF (pfΔ3) cDNA expression vectors. The N terminus of mLRH-1 is shown at the top with four methionine codons in their nucleotide context: nucleotides in bold type match the optimal translation initiation sequence **gccGCCACC**(atg)**Gat** defined by Kozak (31). Vector pf1 expresses mLRH-1 with a modified base context at the first AUG codon. Potential initiation codons (arrows), a peptide segment used to raise anti-FTF antibodies (Ab), and the DBDs (with hatched A and T boxes), receptor homology regions II and III, and *trans*-activatory domain AF2 with its core motif LLIEML (3) (boxes) are indicated. (C) Band shift assays using the FTF probe as in panel A and nuclear extracts from mouse liver or HeLa cells transfected with pf1, pf4, or parental vector pSG5, or reticulocyte translation extracts primed with pf1, pf4, pfΔ2, or pfΔ3. Lanes C, reaction without competitor; lanes FTF and FTFm, 20-fold excess of cold oligonucleotides; lanes 37°b and 37°a, translation products heated at 37°C for 3 min before and after incubation with the FTF probe, respectively. Note the higher proportion of the slower FTF band with vector pf1 than with native mouse liver, referrable to the better translation start site at the first AUG codon in pf1. (Some differences of mobility among FTF variants, for instance lanes 11 versus 20, reflect slight variations between electrophoretic runs.) (D) Dissociation constants of native FTF isoforms 1 or 3 from adult or 4-day-old rat liver, and FTF isoform 3 translated in vitro from the mouse pf1 vector.

at the exact same position as the FTF triplet from mouse liver (Fig. 6C, lanes 5, 11, and 20); vector pf4 (truncated of the first three methionine) yielded a single component comigrating with the fastest hepatic band (Fig. 6C, lanes 8, 14, and 23), and C-terminus-truncated vector pfΔ2 gave three translation products moving slightly faster than pf1 bands (Fig. 6C, lane 17). These results clearly indicated that the three mouse FTF isoforms (and presumably the four rat isoforms) were generated in the protein N-terminal domain by alternative usage of several translation initiation codons. The reticulocyte results provided no indication that FTF might bind DNA as a homodimer (dimers between size isoforms would yield a blurred rather than discrete band shift pattern) and showed that no cell-specific ligand was required for FTF to bind DNA. This concurs with many studies, indicating that orphan receptors generally bind as monomers to nonameric DNA recognition sites, probably unliganded or with ubiquitous ligands (19, 50, 64, 68).

The relative affinities of native FTF isoforms for their AFP binding site were measured by Scatchard analysis on the four rat FTF variants fractionated by electromobility shift. Dissociation constants fluctuated around 0.3 nM for all bands in several adult liver preparations, and similar K_ds were obtained with newborn rat liver (Fig. 6D). Thus, FTF isoforms seem functionally equivalent in binding to their natural target site, and there is no evidence that their affinity may change with liver developmental growth state. The affinity of FTF is in the range of another FTZ-F1 relative, the silkworm BmFTZ-F1 orphan receptor (64). K_d measurements on FTF reticulocyte translation products (pf1 isoforms [Fig. 6D]) differed significantly ($\simeq$1.3 nM) from liver FTF, suggesting that FTF in vivo may undergo posttranslational modifications to enhance its DNA-binding activity: heteromerization with an accessory factor is one attractive possibility because FTF is known to bind the AFP promoter cooperatively with its coactivator LF (7); a preformed FTF-LF complex might dissociate in electrophoretic band shift assay and go undetected in liver nuclear extracts. BmFTZ-F1 is one such receptor that seems to use an accessory protein to enhance its binding to DNA (39). FTF reticulocyte products from vectors pf4 and pfΔ3, which had the N-terminal or N- and C-terminal domains truncated, had K_ds similar to pf1 (data not illustrated): the DNA-binding activity of the FTF DBD segment would thus seem intrinsically independent of the extra-DBD segments. However, further analysis of FTF deletion mutants strongly implicated the C-terminal domain in the DBD function. As previously reported with liver nuclear extract (7), FTF at 37°C loses rapidly and irreversibly its capacity to bind DNA, whereas a preformed FTF-DNA complex is highly stable at 37°C (for an example, see Fig. 9C, lanes 17 and 18). FTF translation products from vectors pf1, pf4, pfΔ1, and pfΔ2 behaved in the same way, but FTF from vector pfΔ3 resisted heat inactivation (compare for instance lanes 21 and 27 in Fig. 6C). This indicated that the DBD heat inactivation domain was located in the C-terminal ligand-binding domain (LBD) between the DBD and region AF2 (activatory function two) (3). LBDs are believed to govern allosteric transitions in receptor structure, unmasking the DBD to contact DNA and the AF2 surface to contact the basal transcriptional machinery (apparently through the short amphipathic AF2 motif LLIEML conserved in many nuclear receptors) (Fig. 6B) (15, 53). Plausibly then, FTF inactivation at 37°C in vitro simply reflects a thermodynamic propensity for the LBD to fold and mask the DBD (some FTF accessory factor, perhaps LF, would then maintain FTF properly unfolded at 37°C in vivo).

***trans*-regulatory effects of FTF or LRH-1.** The preceding results indicated convincingly that all liver proteins bound to AFP *cis*-activatory element FTF were encoded by one mRNA, cloned as rFTF or mLRH-1. Therefore, a correlation between FTF binding and AFP promoter activity forcefully predicted that one or more FTF isoforms from vector pf1 should activate the AFP promoter. Cotransfection assays were designed to address the site of FTF action and its proper interaction with coactivators HNF-1 and LF. The first set of experiments used HepG2 cells with AFP-CAT construct 5200, carrying the full AFP enhancer and promoter regulatory domain (Fig. 7A). Cotransfection of 5200 with vector pf1 enhanced AFP-CAT activity by a factor of 3 (Fig. 7A, lane 2), thereby meeting a *trans*-activatory effect expected for FTF. Vector pf4, encoding the short FTF isoform (Fig. 6C), had essentially the same effect (Fig. 7A, lane 3), indicating no need for an N-terminal *trans*-activatory domain (3). C-terminus-truncated FTF from vectors pfΔ3, pfΔ2, and pfΔ1 repressed AFP-CAT activity to the same extent as glucocorticoid hormones, which completely suppress AFP gene transcription in vivo (62) (Fig. 7A, lanes 4 and 7). C-terminus-truncated FTF thus behaved as a dominant negative mutant, and it narrowly mapped an essential *trans*-activatory function surrounding element LLIEML in domain AF2, as in ligand-dependent receptors (15). As anticipated, wild-type or mutant FTF had no significant effect on SV40 or albumin promoters, which contain no receptor recognition sites (Fig. 7A, lanes 12 and 16). Other AFP enhancer and promoter constructs confirmed that the FTF response element was located in the upstream promoter: construct E4-202 with an intact AFP promoter was suppressed more than 10-fold by pfΔ3, whereas E4-158 truncating the FTF binding site (and accordingly far less active than E4-202) responded less than 1.3-fold (Fig. 7A, lanes 8 to 11). As a stringent test for FTF function, we also used three copies of the AFP segment from nucleotides −171 to −114 in front of a minimal promoter (mUP-TK40 [Fig. 7B]). The AFP segment from nucleotides −171 to −114 forms a discrete composite enhancer (7, 25) strictly dependent on all three binding sites for FTF, LF, and HNF-1, and it is deactivated by glucocorticoid hormones, as in its natural promoter context (7, 22). Coexpression assays made use of HeLa cells, which do not express HNF-1 or FTF. Cotransfection of HNF-1α and/or FTF had no effect on a basal TK-CAT construct (Fig. 7B, lanes 9 to 13), and either factor alone had only a marginal effect on mUP-TK40 (Fig. 7B, lanes 2 and 3). However, when HNF-1α and FTF were transfected together, a synergistic effect was observed with mUP-TK40, raising basal promoter activity more than 30-fold (Fig. 7B, lane 5). Moreover, the effect was antagonized by FTF mutant pfΔ3 and by GRs (Fig. 7B, lanes 7 and 8), thus mimicking entirely the regulated behavior of the AFP gene in hepatic cells.

The proper control of the FTF-LF-HNF-1 domain in HeLa cells, solely by expression of FTF and HNF-1, indicated that coactivator LF was not liver restricted. The DNA-binding cooperativity of FTF with LF (7) (see also Fig. 9C, lanes 15 and 16) and the fact that FTF lacked an N-terminal activatory domain (AF1) (3) then suggested the possibility that FTF might be an incomplete *trans*-activator using a ubiquitous accessory factor (LF), perhaps to keep the DBD and AF2 domains in adequate configuration or to establish a composite activation surface. This was tested with CAT constructs using the FTF-binding AFP element and adjacent sequences in front of heterologous promoters (Fig. 7C). One copy of the FTF binding site failed to activate the 105-bp TK promoter in HepG2 cells; however, one copy of the tandem FTF-LF segment elicited a consistent twofold enhancement of TK-CAT activity, repressible by glucocorticoid hormones (Fig. 7C, lanes 2 and 3). These results were thus consistent with FTF having to interact with LF in order to interface the proximal promoter.

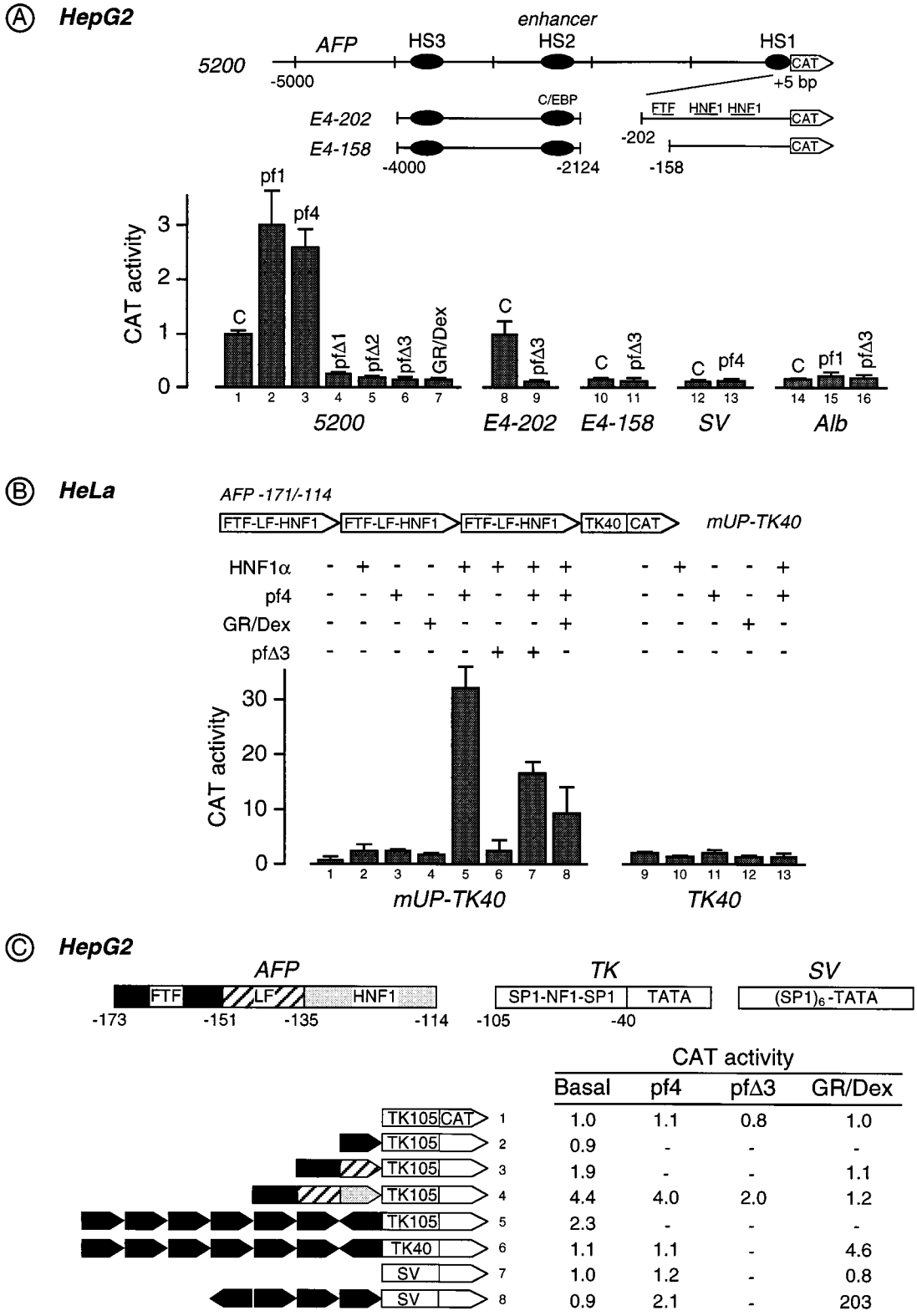

FIG. 7. AFP regulatory effects of mLRH-1 and rFTF cDNA expression vectors in transfection assays. (A) HepG2 assays with AFP-CAT reporter constructs carrying the three DNase I-hypersensitive sites (HS1 to HS3) found in AFP-active liver chromatin (62). Control SV-CAT carries the SV40 promoter in $pA_{10}cat_2$ (32), and Alb-CAT carries rat albumin promoter region from nucleotides −160 to +3 in pBluescript SK^+. Expression vectors pf1, pf4, pfΔ1, pfΔ2, and pfΔ3 (Fig. 6B) were tested at the same concentration (10 μg) in parallel with control parental vector pSG5 (bars C), and CAT activities (normalized for transfection efficiency) (7) were expressed relative to control 5200 given an arbitrary value of 1. GR-Dex, 2 μg of human GR expression vector with 1 μM dexamethasone (Dex) (GR or Dex alone had no effect). Results are averages from four triplicate transfections; error bars show 1 standard deviation. (B) Assays using an AFP-CAT construct with three 5′→3′-oriented copies of the upstream AFP promoter domain fused to a minimal TK promoter (from nucleotides −40 to +51). mUP-TK40, or control vector TK40-CAT, was cotransfected in HeLa cells with 10 μg of cDNA expression vectors (except for GR [2 μg], with 1 μM Dex). CAT activities are in arbitrary units relative to reporter construct alone; results are averages ± standard deviations from three triplicate transfection assays. (C) HepG2 assays with segments of the FTF-LF-HNF1 region linked to the 105- or 40-bp TK promoter or to the SV40 promoter in CAT constructs. Transfection was done as described above for panel A, with basal levels obtained from control vector pSG5. CAT activities are relative to basal TK105-CAT. Results are averages of three highly reproducible transfections.

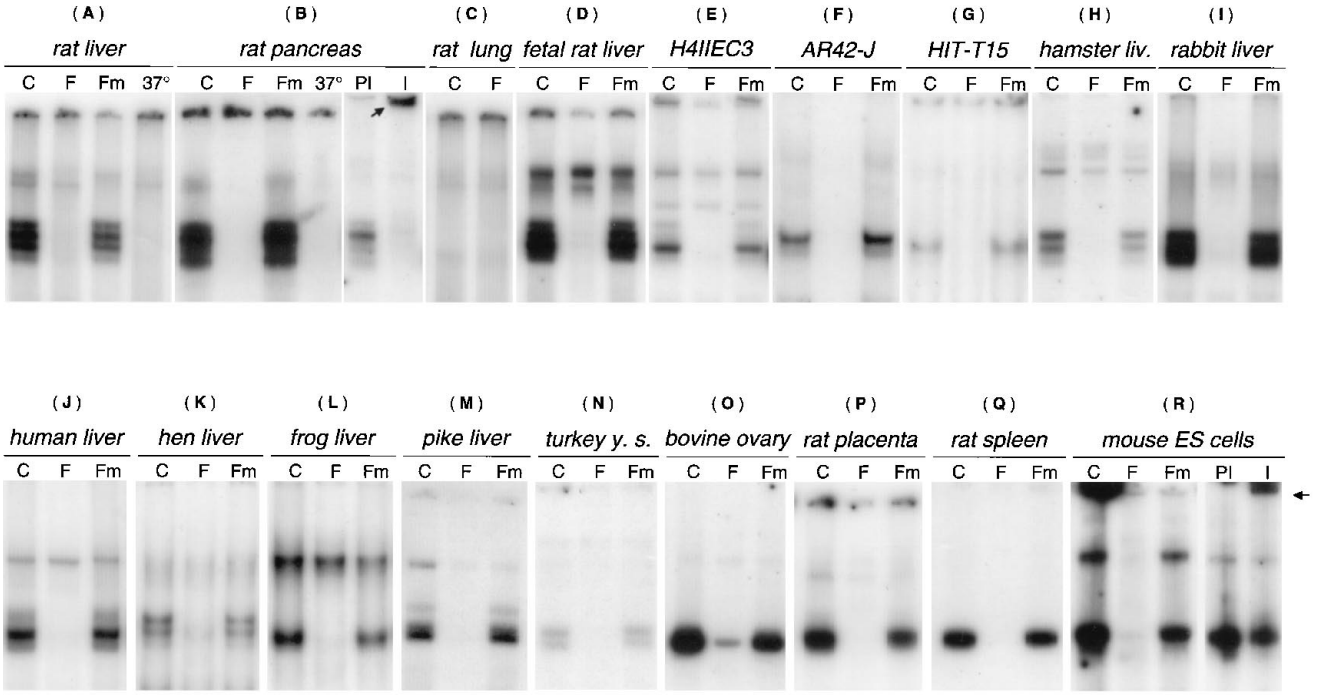

FIG. 8. Electromobility shift and supershift assays using FTF probe and antibodies as described in the legends to Fig. 2D and 5. Nuclear extracts (2 to 5 μg) were used. Lanes: C, no competitor; F, and Fm, 10- and 20-fold excess of cold oligonucleotides; 37°, extract preheated for 3 min at 37°C; PI, preimmune rabbit serum; I, rabbit anti-FTF serum. AR42-J is a rat pancreatic cell line, HIT-T15 is a hamster pancreatic cell line, ES are embryonic stem cells, and H4IIEC3 is a rat hepatoma line. Arrows mark supershift complexes. y.s., yolk sac.

Adding a contiguous HNF-1 segment added only twofold enhancement (Fig. 7C, lane 4), far from the synergistic effects delivered in native chromatin; thus, up-regulation by FTF also appeared strongly dependent upon promoter context. The FTF sequence was further assayed in multimeric form, one advantage here being that multiple receptor half-sites created a response element to GR, a complete transcriptional activator (3, 58). When multimerized upstream of TK105, the FTF sequence did enhance promoter activity two- to threefold (Fig. 7C, lane 5), suggesting that FTF had some intrinsic *trans*-activating potential with a complete promoter. However, in front of minimal promoter TK40, the FTF multimer had no effect, whereas GRs achieved ≈5-fold enhancement (Fig. 7C, lane 6). Thus, while FTF appears to interact positively with other upstream transactivators (SP1 and NF1 in TK105), its AF2 domain alone apparently cannot mount a productive interface with general transcription factors; in contrast, GRs, with both AF1 and AF2 domains, can establish autonomous contacts with a basal promoter (58). An FTF binding-site tetramer fused to the SV40 promoter also produced only low induction by FTF; glucocorticoid induction was 100-fold higher (Fig. 7C, lane 8). These combined results seem to support the view that receptor FTF alone is a weak or incomplete *trans*-activator, distinct from receptors that use both AF1 and AF2 activatory functions. This may explain the need for a DNA-bound FTF coactivator.

Histotypic and phylogenetic distribution of FTF. With conclusive data to link orphan receptor FTF with at least one target gene, AFP, we looked for FTF in the tissues and cells of different species, lineages, and developmental stages to gain further insights into AFP gene regulation and perhaps other FTF functions. In adult rats, we found that FTF was as abundant in the pancreas as in the liver (Fig. 8B) and negative in all other tissues tested. These tissues included ectodermal and mesodermal organs (brain, thymus, muscle, blood, fat, and kidney) but also other endodermal derivatives such as lung and intestine (Fig. 8C), which express mRNAs for other liver-type transcription factors (HNF-1, C/EBPα, HNF-3α-β, and HNF-4) (13, 57, 59). The FTF distribution pattern was the same in neonatal rat tissues, and FTF was also strongly expressed in fetal liver (Fig. 8D), indicating particularly efficient use of its low-abundance mRNA (Fig. 4). Further analyses conducted with hepatic and pancreatic rodent cell lines showed that FTF is present in AFP-permissive (e.g., 7777 rat cells) as well as in AFP-nonpermissive (e.g., rat H4IIEC3) hepatoma cells (Fig. 8E and 9A) and in pancreatic parenchymal cells both of exocrine (rat AR42-J) or endocrine (hamster HIT-T15) origins (Fig. 8F and G), similar to HNF-1 (59). Liver FTF was also detected in all tested vertebrates (Fig. 8H to M), up to fish (Fig. 8M) and including avian liver (Fig. 8K), which never expresses AFP (56) (FTF was found in avian yolk sac, which produces AFP [Fig. 8N] [56]). As expected, the AFP FTF probe detected a specific component (different from FTF by supershift assays) in rat, mouse, and bovine testis, ovary, and placenta (Fig. 8O and P), corresponding to the distribution of SF-1. One organ, the spleen, yielded a specific component different from FTF and SF-1 (Fig. 8Q) (24, 44); the spleen component could be ELP, the alternative product from the SF-1 locus, since PCR primers used by Honda et al. (24) to exclude SF-1 in spleen would have missed the ELP transcript. Among all cells tested, only mouse embryonic stem cells yielded a mix of FTF (supershifted) with another band shift component, moving as SF-1 or ELP (Fig. 8R). Finally, HepG2 cells contain an abundant FTF site-specific factor (Fig. 9A, lanes 1 to 3), with a K_d similar to that of FTF (Fig. 9B); but in contrast to FTF from human liver or rat hepatoma cells, the

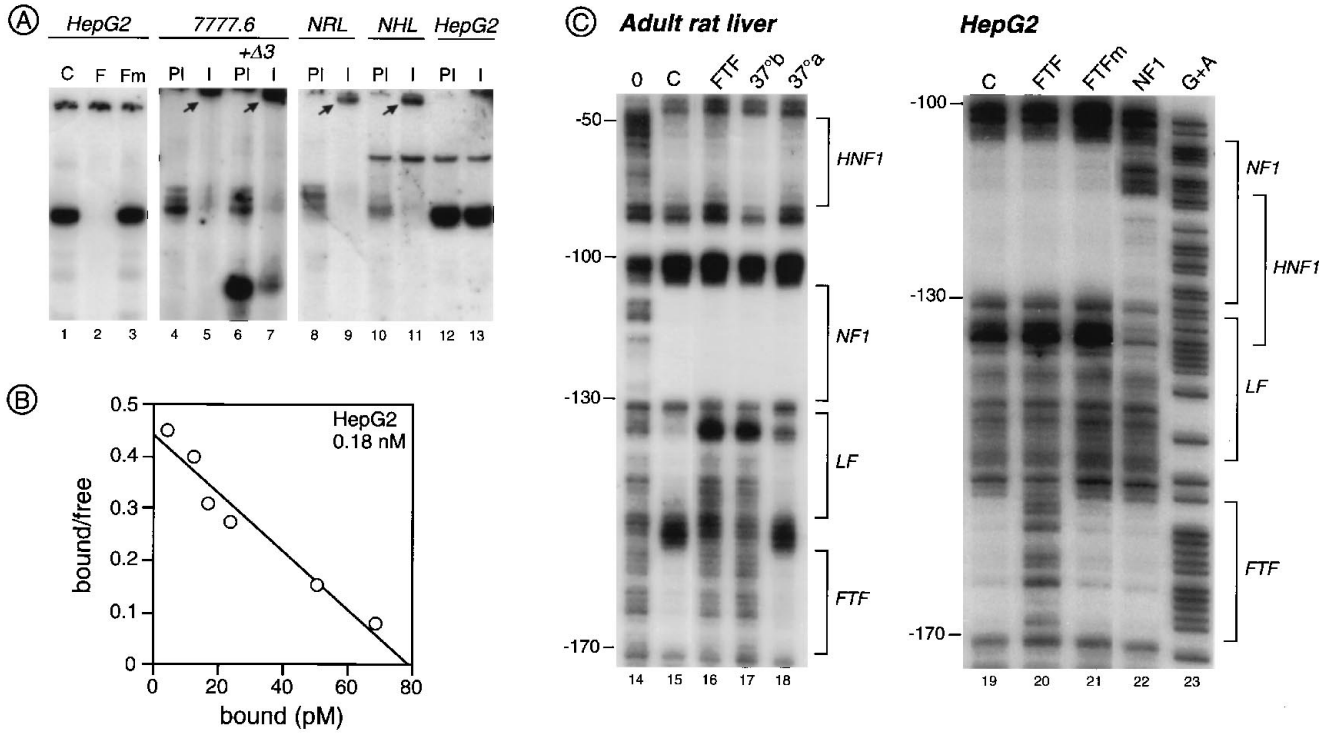

FIG. 9. Characterization of an FTF-related factor in human HepG2 hepatoblastoma cells. (A) Electromobility shift and supershift assays conducted as described in the legend to Fig. 8 with 2 μg of nuclear extract from HepG2 cells in exponential growth, normal rat liver (NRL), normal human liver (NHL) and 7777.6 rat hepatoma cells that are wild type or stably transfected with rat FTF deletion mutant pfΔ3 (+Δ3). Arrows point to supershifted FTF complexes. (B) Scatchard analysis of FTF site affinity of the HepG2 factor, with a calculated K_d similar to that of FTF (Fig. 6D). (C) DNase I footprinting assays (7) using 1 ng of rat AFP promoter fragment from nucleotides −244 to −15 (radiolabeled on the noncoding strand) and 60 μg of nuclear extract from rat liver or HepG2 cells. Lanes 0, DNase I reaction without nuclear protein; C, 37°b and 37°a, reactions kept at 0°C (C) or heated for 3 min at 37°C before (37°b) or after (37°a) adding the probe; FTF, FTFm, and NF1, 500-fold excess of AFP promoter segment from nucleotides −103 to −127 (NF1) or 200-fold excess of oligonucleotides FTF or FTFm; G+A, chemical cleavage reaction. Note that the FTF oligonucleotide displaces both the FTF and LF liver footprints, indicating that FTF recruits LF on DNA. Also note the stabilization at 37°C of both FTF and LF prebound to DNA, and the weak LF protection pattern with the HepG2 extract compared with normal liver.

HepG2 factor is not supershifted by anti-FTF antibodies (compare lanes 5, 11, and 13 in Fig. 9A) and in DNase I footprint assays, the HepG2 factor cooperates poorly with FTF accessory factor LF (compare lanes 15 and 19 in Fig. 9C). Ectopic expression of ELP seems excluded, since ELP expression vectors drastically suppress AFP-CAT transcription in HepG2 cells (our unpublished results); ectopic expression of SF-1 concomitant with extinction of the FTF locus also appears unlikely. This seems to favor the interpretation that HepG2 cells express a different form of FTF.

DISCUSSION

The albumin paradigm of liver chromatin function reaches broadly into developmental and homeostatic events molding the hepatocyte phenotype. Among albumin regulatory domains, the AFP promoter has been targeted for its early activation in hepatocyte progenitors and its reactivity to growth and hormonal signals, suggesting a tight connection with high-ranking liver differentiation factors. A key regulator of the AFP promoter is identified here as an orphan nuclear receptor previously detected in several vertebrates, but so far without a defined function. rFTF and its rodent mLRH-1 (61), primate PHR-1 (4), and amphibian xFF1rA (18) orthologs constitute after SF-1 the second vertebrate receptor clustering with *Drosophila* FTZ-F1, involved in insect metamorphosis (36, 37). The first target gene assigned to FTF thus also has an early developmental function, exerted in a narrow hepatopancreatic lineage, as for SF-1 in steroidogenic compartments.

The question then arises as to whether FTF operates as a liver-constitutive factor like HNF-1α and C/EBPα, or if FTF is a modular switch controlling hormonal, developmental, and metabolic interplays at the AFP promoter. During liver development, AFP transcription declines with liver growth and can be prematurely deactivated by glucocorticoid hormones; with hepatoma cells in culture, "paradoxical" repression of AFP can also be observed following stimulation of growth pathways, notably the protein kinase C signal transduction pathway (8, 46). Many of these effects, particularly AFP repression by GR (7, 22) (Fig. 7B and C), Ras (46), or Jun (7, 8), have been circumscribed to the FTF-driven upstream promoter, using isolated transfection systems. Plausibly then, AFP promoter function could obey FTF modifications connected with cell growth or metabolic signals. However, as attractive as a computational element can be, we gather no evidence that FTF (or its accessory factor LF) can qualify as a metabolic or growth sensor: band shifts, footprints, K_ds, and chromatographic behavior on heparin-Sepharose are all but indistinguishable in fetal, newborn, and adult livers (Fig. 6 and 8) (7). FTF distribution in distant species, its activity in HeLa cells, and its presumptive activatory properties in adult liver or pancreas also argue against a cell- or stage-specific ligand or posttranslational modifications through which FTF might induce or deactivate AFP. The noninducible character of FTF also contrasts sharply with that of a growth-inducible orphan receptor like NGFI-B (23) and would seem to fit more with the liver-constitutive behavior of the albumin multigene cluster, react-

ing to broad homeostatic movements like plasma colloid osmotic pressures. We thus favor the simple view that FTF is a constitutive ligand-independent transcriptional activator, recruited long ago at the albumin gene cluster as an abundant, lineage-specific factor needed to activate AFP at early stages of development when alternative activators C/EBP and HNF-1 are at a low level. How then might we rationalize cross-talks between FTF and GRs or Ras and Jun? The answer may reside in the modular structure of nuclear receptors and the tight phylogenetic conservation between DBD modules of GR and FTZ-F1 receptors (35). Glucocorticoid repression of AFP is strictly dependent upon the GR DBD (63), and as we will report elsewhere, AFP repression probably occurs by competitive protein-protein interaction with the FTF DBD and FTF accessory factor LF. Ras or Jun repression of AFP could perhaps similarly proceed from FTF–AP-1 interactions as documented for GR–AP-1 (3). It will thus be interesting to see if GR-FTF cross-talk may also apply to other receptors sharing closely related DBDs, particularly SF-1 and the GR-related receptors. At least one promoter seems to use SF-1 with an adjacent coactivator repressible by glucocorticoid hormones, similar to the FTF-LF complex (11, 27).

In the search for an FTF regulation domain, it may be particularly revealing that FTF is selectively expressed in the liver and pancreas, coderivatives of the gut endoderm (10, 38), while no FTF-binding sites could be found in pancreatic genes exerting mature endocrine or acinar functions. FTF recognition sequences in transferrin and α_1-antitrypsin further suggest that FTF might control several gene functions needed for early developmental growth. Yet more provocative is the FTF site in the HNF-3β gene (51), which governs differentiation in the endodermal lineage (1, 66). The pressing hypothesis would then seem to be that FTF primarily controls early developmental cell fates and gene products in the hepatopancreatic lineage. Primitive characters of FTF, phylogenetic and structural, can also argue for FTF being an ancestral developmental regulator.

FTF control of the archetype carcinoembryonic gene raises further intriguing ways in which FTF might be involved in aberrant differentiation leading to neoplasia. Aside from possible FTF interference with GR maturation pathways of hepatocytes, it is tantalizing that HepG2 hepatoblastoma cells contain an FTF-related factor, probably the same as factor PCF (67) and different from liver FTF. FTF might perhaps exist as splice variants with different functions, like SF-1 and ELP in mammals (26, 48, 60), early and late FTZ-F1 in *Drosophila melanogaster* (36), or C-terminus-truncated FTF in *Xenopus laevis* (xFF1rAs in reference 17); or like other nuclear receptors that qualify as bona fide oncogenes, FTF might also contribute to tumor progression as a mutated, truncated or fusion gene product. It is also noteworthy that the hepatitis B virus promoter contains two FTF-binding elements (40).

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