Molecular cloning of the mammalian fatty acid synthase gene and identification of the promoter region

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Rat genomic clones encompassing the entire fatty acid synthase gene have been isolated and characterized. The gene is present in a single copy of approx. 20 kb. Genomic DNA sequencing, direct RNA sequencing and S1 nuclease analysis showed that transcription is initiated primarily 1274 nucleotides upstream from the translation start site and that the 87-nucleotide-long 5'-untranslated mRNA sequence is the same in liver, lung and mammary gland. The 5'-flanking region and first intron contain several sequence elements which may be involved in the transcriptional regulation of this gene.

INTRODUCTION

In mammals, the pathway of fatty acid biosynthesis *de novo* is regulated by dietary, developmental and hormonal influences in a tissue-specific manner. Lipogenesis in liver and adipose tissue is intimately connected with maintenance of the energy balance of the organism, providing for conversion of excess carbohydrate into storage lipid. In maturing fetal lung, the pathway generates the palmitic acid required for biosynthesis of lecithin, an essential component of surfactant [1], and during lactation the mammary gland lipogenic system supplies the medium-chain fatty acid of milk fat [2]. In all cases where lipogenesis *de novo* has been shown to be altered, fatty acid synthase activity in the respective tissues is affected and changes in the amount of fatty acid synthase protein are accompanied by changes in the amount of fatty acid synthase mRNA present [3–5]. These findings strongly suggest that fatty acid synthase is regulated at the level of transcription.

As a first step in elucidating the mechanisms regulating transcription of the fatty acid synthase gene, we report here estimation of the gene copy number, identification of the transcriptional start site, the sequence of the 5'-flanking region of the gene and the location of several potential transcriptional regulatory elements.

MATERIALS AND METHODS

Gene copy number analysis

Portions of spleen DNA (10 mg) isolated from Sprague-Dawley rats [6] were digested with restriction enzymes, electrophoresed on 0.8% agarose gels, transferred to Nytran membranes and immobilized to the support by u.v. cross-linking. Probe DNA, labelled with [α -³²P]dCTP (3000 Ci/mmol) by random priming, was hybridized to DNA blots in 0.25 M-Na₂HPO₄/0.25 M-NaH₂PO₄/0.5 M-EDTA/7% SDS/1% BSA at 65 °C for 18 h. Blots were washed in 2 × SSC/0.1% SDS at room temperature, then in 0.5 × SSC/0.1% SDS at 55 °C prior to radioautography. For the estimation of gene copy number [7], DNA was digested completely with *Sac*I, mixed with various amounts of a 1.57 kb *Eco*RI/*Sac*I restriction fragment derived from plasmid pFAS 5 [8], electrophoresed in a 1% agarose gel

and transferred to a Nytran membrane. A 392 bp *Hpa*I/*Sac*I restriction fragment from pFAS 5 was labelled with [α -³²P]dCTP by random priming and hybridized to the DNA blots under standard conditions [9].

Genomic clone isolation and sequencing

Genomic libraries (obtained from Dr. C. Glockin, Phytogen Corp., Pasadena, CA, U.S.A.), prepared from partial *Eco*RI or *Hae*III digests of Sprague-Dawley rat DNA cloned into the *Eco*RI site of the λ vector Charon 4a [10], were screened with probes containing parts of rat fatty acid synthase cDNA clones obtained from a lactating rat mammary gland library [11]. Double-stranded genomic DNAs recloned in pUC vectors were sequenced using [α -³⁵S]dATP (3000 Ci/mmol) and the T7 Sequencing Kit (Pharmacia) according to the manufacturer's instructions.

Northern-blot analysis

Total RNAs, isolated from Long-Evans rats [12], were electrophoresed (10 μ g per lane) on 2.2% formaldehyde/1% agarose denaturing gels [13], transferred to Nytran membranes overnight with 10 × SSPE, and immobilized by u.v. cross-linking. Labelled probe DNA was hybridized to the immobilized RNAs in 50% formamide/5 × SSPE/1 × Denhardt's solution/0.1% SDS/yeast tRNA (100 μ g/ml) at 42 °C for 18 h. The membranes were washed four times for 15 min each at room temperature with 0.2 × SSPE/0.1% SDS [9]. Oligonucleotide probes were end-labelled with [γ -³²P]ATP (> 7000 Ci/mmol) and polynucleotide kinase and hybridized overnight at 42 °C to blotted RNAs in a solution containing 6 × SSPE/1 × Denhardt's solution/yeast tRNA (100 μ g/ml)/0.05% sodium pyrophosphate. The membranes were then washed four times for 15 min each in 6 × SSPE/0.1% sodium pyrophosphate at room temperature, then again at 37 °C for 30 min, and radioautograms were prepared.

RNA primer extension and sequencing

An end-labelled oligonucleotide complementary to the rat fatty acid synthase cDNA sequence extending 30 bases upstream from and including the ATG translation start codon was hybridized to 50 μ g of total RNA in 30 μ l of 80% formamide/

Abbreviations used: 1 × SSC, 0.15 M-NaCl/0.015 M-sodium citrate, pH 7.0; 1 × SSPE, 0.18 M-NaCl/0.01 M-sodium phosphate buffer (pH 7.7)/1 mM-EDTA; FSE, fat-specific element.

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The nucleotide sequence data reported here will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number X54671.

0.04 M-Pipes buffer (pH 7.2)/0.4 M-NaCl/1 mM-EDTA at 30 °C for 18 h and extended with 32 units of avian myeloblastosis virus (AMV) reverse transcriptase, 30 units of RNasin (Promega) and 0.5 mM-dNTP per 25 μ l reaction [14]. Samples were incubated for 1 h at 42 °C, treated with RNAase, extracted with phenol/chloroform (1:1, v/v), precipitated with ethanol and analysed on a 6% polyacrylamide/7 M-urea sequencing gel.

For direct RNA sequencing, primer extension reaction mixtures containing 12 μ M of each dNTP were modified to include 25 μ M-ddATP, -ddCTP or -ddGTP or 12 μ M-ddTTP for a 15 min incubation at 42 °C [15]. The dNTP concentrations were then increased to 150 μ M, incubation was continued for an additional 45 min at 42 °C and the reaction products were identified as described above.

S1 nuclease analysis

The probe for S1 nuclease analyses, containing the genomic sequence (non-coding strand) from nucleotides -118 to +73, was prepared by primer extension. An end-labelled oligonucleotide complementary to the sequence from +57 to +73 on the coding strand was annealed to a subclone of rg 27-22 (denatured in 0.2 M-NaOH) which included the genomic sequence from about -4083 to +1540. Next, dNTPs (0.4 mM) and 10 units of the Klenow fragment of DNA polymerase were added to extend this primer in the 5' to 3' direction. The products of this reaction were digested with *Sma*I and electrophoresed on a 1.2% low-melting-temperature agarose gel in an alkaline denaturing buffer. The 191-nucleotide-long single-stranded DNA fragment was cut from the gel, extracted with phenol and concentrated by ethanol precipitation.

This probe DNA was hybridized to 50 μ g portions of total RNA for 10 min at 65 °C, then for 18 h at 30 °C in the same solution used for the primer extension experiments described above. The mixture was digested with 300 units of S1 nuclease in 300 μ l of 0.28 M-NaCl/0.05 M-sodium acetate buffer (pH 4.5)/4.5 mM-ZnCl₂ for 60 min at 37 °C [16]. The reaction products were concentrated, electrophoresed on sequencing gels and detected by radioautography.

Fig. 1. Determination of rat fatty acid synthase gene copy number

(a) Southern blots of rat spleen DNAs digested with restriction enzymes (lane 1, *Eco*RI; lane 2, *Xba*I; lane 3, *Bgl*II; lane 4, *Bcl*I) were prepared and hybridized with the labelled insert from cDNA clone pFAS 27. Approximate sizes (in kb), based on the migration of DNA standards, are indicated on the left. (b) Graph showing the gene copy number as determined by comparison of the hybridization intensities of 1.04, 2.07 and 3.11 pg of the *Eco*RI/*Sac*I fragment of pFAS 5 (●) with those obtained from *Sac*I-digested rat spleen DNA (○) using a 392 bp *Hpa*I/*Sac*I probe.

RESULTS AND DISCUSSION

Gene copy number determination

Southern analysis with a probe from the 3'-end of the fatty acid synthase cDNA revealed a single positive species in rat genomic DNA digested with *Eco*RI, *Bcl*I or *Bgl*II; only *Xba*I-digestion generated two positive bands (Fig. 1a). These results

Fig. 2. Map of rat fatty acid synthase genomic clones

Clones (represented by solid bars) from two rat genomic libraries overlap as indicated and are displayed below a partial restriction enzyme map. Vertical arrows mark the 5'- and 3'-ends of the mRNA for fatty acid synthase; the location of the 392 bp *Hpa*I/*Sac*I restriction fragment used for gene copy number determination is indicated by a filled box at 16.8 kb. The approximate position of the *Eco*RI site at the 5'-end of the map about 23 kb upstream from the internal *Eco*RI site is based on Southern-blot analysis (see Fig. 1). The expanded map at the top of the Figure shows the region surrounding the transcription start site including the untranslated (open boxes) and translated (shaded box) parts of the first two exons. Horizontal arrows below this map indicate the direction and extent of the individual overlapping sequencing reactions used to derive the sequence presented in Fig. 5.

Fig. 3. Primer extension, sequencing and S1 nuclease analysis of RNAs

(a) The primer-extended products of the RNA from spleen (Sp) and from liver (Lv) isolated from rats fasted for 48 h and then fed on a fat-free diet for 24 h are shown; the lengths of the products were determined by comparison to pFAS 27 DNA sequenced using the same primer (Std). (b) Liver RNA was the template for the sequencing reactions, and the deduced sequence from +3 to +45 in the non-coding direction is shown on the right. Arrows mark the positions of the most abundant product (nucleotide +1 of the genomic sequence) and of the longest of the apparently less-abundant upstream species (-5). (c) The products of S1 nuclease digestion of hybrids formed between RNA isolated from lactating rat mammary gland (MI) or from the livers of fasted then refed rats (Lv) and the single-stranded probe DNA are shown. The sizes of these products and of the undigested DNA probe (Pr), measured in nucleotides from the 5'-end of the probe, are shown on the right.

suggested that the gene occurs in a single copy; confirmation was obtained by quantitative analysis. A 392 bp cDNA probe, corresponding to part of a 2.04 kb *SacI* genomic restriction fragment which is uninterrupted by introns, was used to compare hybridization signals between the 1.57 kb *EcoRI/SacI* pFAS 5 cDNA fragment and the rat genomic *SacI* fragment. Thus, since the rat haploid genome is 3×10^6 kb in size [17], 2.07 pg of the cDNA would be expected to produce the same hybridization signal as 3.96 μ g of genomic DNA. The data (Fig. 1b) showed that there are 1.00 ± 0.07 (mean $\pm$ S.D., $n = 4$) fatty acid synthase gene equivalents per haploid rat genome.

Genomic clone characterization

First, a clone (rg 5-3) with a 16 kb insert partially identical with both cDNA clones 3 and 5 was isolated from a rat genomic *EcoRI* library. Restriction enzyme and sequence analysis showed that the 5'-end of rg 5-3 corresponded to the unique *EcoRI* site in the cDNA sequence and that the 3'-end of this clone extended beyond the 3'-untranslated end of the cDNA (Fig. 2). No positive colonies could be identified using a probe from the 5'-end of the cDNA, presumably because the *EcoRI* fragment encompassing the 5'-end of the gene was too long to be cloned

Fig. 4. Northern analysis of fatty acid synthase mRNA from different tissues

(a) RNAs (10 μ g) isolated from brain (Br), lung (Lu), spleen (Sp), mammary gland of pregnant (Mp) and 4-day-lactating (MI) rats, and from the livers of fasted, then refed rats (Lv) were electrophoresed, blotted and hybridized to the labelled insert from pFAS 27. Oligonucleotides complementary to the cDNA sequence of nucleotides 29-45 (b) and to the genomic sequence of nucleotides 13-22 (c) were used as probes for duplicate blots of the lactating mammary gland and liver RNAs probed in (a). The location of the 8.3 kb and 9.1 kb fatty acid synthase mRNAs are indicated.

into the λ Charon 4a vector used to construct the genomic library (Fig. 1a).

Screening of a library constructed from *HaeIII* partial digests of rat genomic DNA yielded numerous plaques positive for clone pFAS 27 which contained a 1.1 kb insert from the 5'-end of the cDNA. Clones rg 27-3 and rg 27-23 each contained an internal *EcoRI* site corresponding to the unique *EcoRI* site at nucleotide 2545 of the cDNA sequence plus 3.75 kb and 7.0 kb respectively of sequence upstream from the *EcoRI* site. A third clone (rg 27-22) had a 13.5 kb insert whose 3'-end was approx. 460 bp from the *EcoRI* site in the genomic sequence (Fig. 2). Thus clones from the two libraries span about 30 kb of contiguous genomic sequence.

Identification of transcription initiation sites

As previously reported [11], clone pFAS 27 isolated from a lactating rat mammary gland library appeared to include all of the 5' non-coding end of the rat fatty acid synthase mRNA. This conclusion was supported by the results of primer extension experiments with RNA from tissue expressing a high level of fatty acid synthase mRNA (liver from fasted, refed rats) compared with RNA from spleen, in which fatty acid synthase expression was not detected (see Fig. 4a). The 87-nucleotide-long product obtained with liver RNA and not with spleen RNA corresponded to the expected size, based on the length of the cDNA clone pFAS 27 (Fig. 3a).

The genomic DNA sequence showed that nucleotides 46-75 of pFAS 27 were separated from the exon containing the translation start site by an 1187 bp intron. However, the first 45 nucleotides of the pFAS 27 sequence were not located within the upstream 6.1 kb of rg 27-22. To resolve whether the 5'-end of cDNA clone pFAS 27 corresponded to the 5'-end of the fatty acid synthase mRNA, we sequenced the fatty acid synthase RNA directly. Analysis of the products of these reactions showed that the fatty acid synthase mRNA sequence corresponded to the genomic sequence and not to the sequence found in pFAS 27 (Fig. 3b). Additional sequencing of RNA isolated from mammary gland and from lung showed the 5'-end of these mRNAs to be identical with that of rat liver mRNA (results not shown).

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-1592      GAGCTCTGCTGA  CCAAGTATGGTTAGCCAGCA  GCCTGGCCAGCGAGAAGGCA  AACACAGGGCACCTGGAGGT  GGGGTGGGACAGCTCTGGCT
-1500  GGCACATGCACAAGCCATTT  ATAAACTCTCTAGGCTGA  CCCTGAACCCCAAAGACACA  ACAAAGTGAAGACCCCTCCA  TAGAGAGAGTGCAGGACCTG
-1400  GGTCCCGCACTCCTCACCTC  CATAGAATACACATTACAAC  AAGAGATGCCATCAGTAGGC  CATGGAACAGGCCCTAAAGA  CACAGGGGCACTAATAGGAG
-1300  AACTTGGGGGCAAGCCGCGCA  TGTGGGCAGAAATACACCTGC  CAGTCTTACAGGCTGCCCTT  CAGGGTCCCCACTTAGCCTC  CTTCCACAGAGAGCCTGTGG
-1200  ACAGCCAAAGCTGGAGAAGCT  AGAAGCCAGGGTTGACAAGC  AAGGCTCTGAGGCTCTGGCT  TTTGCTATAGACATGCAGTC  AAGGACCACTGACACTACTC
-1100  CCTCCTTAGCGCCCCCGCC  CCCAAAGCCACTGCCCATAA  GGTTGGTCTTAGTGCCCTGG  CCCTGTAGTGAAGGGCAGA  AGGAAAGGGATCAACTCTGA
-1000  GCAGTCTGTGTCTTTTGGT  CGGTGAGTTTTCATCATCTC  CCGTCCCAAAATTCGATAAC  CCTTTCAAAGAGGAATTTA  AAGGGAGGGAGGGTGAAGGT
-900  CCCGGAACACAGCAACTCAG  GGAGGCGCGCAGACGCTCTT  TGTTCACACAGGCGGGGAG  GGTGGTATCCCGCTCGCCAG  ATGGCCGCGCTGGACACTG
-800  AACGGACTCAGGAGACCGCG  GCACGCGCCGCTCAGTGTTC  CCTATCTGCCTACTGCTCT  CGTCCCTGCCCGCATCTGG  TCTCAAGGCGGCCACAGAA

      ERE/TRE
-700  AGGGTGGGTGTCTGAGAAAG  CTGGCCACGATGACCGGTA  GTAACCCCGCCTGAGGCGCC  CTCCGCCAGGGTCAACGACC  GCCTTGC CGCGGGGGCCCGA

-600  GAAGCGCTTTGCCGCTTTTG  CCGGCCCATCACCTATTGC  CTAGCAACGCCACCGCGCG  GCCACCATTTGGGCCACCGAG  AACGGCTCGGTGTCCAATT

-500  GGTCTCGATGTGGAGCAGGC  CACGCCCTCGGCTCTGCGC  GCGTACGATCAGCGGTGG  AATCGCAGCGACACGGACCT  GTTCTGTTCCCCCGGTGGC

-400  CCCAGTGTCTCAGTGCAGT  TCCAGTGTGACCAAGCAGC  CCGACCCACACTGCGCGCG  ACAGTGCACACTGGCCCCG  GCCGCGGGGTGGGGGGTG

-300  AGAGAAAGGACAGAGATGAG  GGCCTCGGATGAGCCCCG  GTGGCCCGGGAGGCGCGG  GGCGGGGACGGAAGCGGGC  GGGGCTGCGGTTCTTGTG

-200  CTCCAGCGCGCGCTGTGCA  GGGTCCCGGCTGGGGCGGC  GCGCGCGGCATCACCCAC  CGACGGCGCGCGCGGGGT  CCGGGGCGCAGCCCCGACGC

-100  TCATTGGCTGGCGCGCA  GCCAAGCTGTACGCCCATGT  GCGTGGCGCGCGGGGATG  GCCGCGCTTTAAATAGCGCC  GGCGCGCCTAGAGGGAGCC

+1  AGAGAGACGCGCAGCGCTC  CCGTCCAGTTCGCTGCGCG  GCTCTCGCTTGTGCTCTGC  CTCCAGATCCCAGACAGTA  CGCCGCTGCGTTCGGGGTTC

+100  CGGGATGGCAGGCGGTGTC  GGGGTGCCGCGGCTCCAGTG  GGAGGACAGGCGGGCGCCA  TCCTCAACAGCCCTGCGCTC  ACAAAGCGCCGCCGCGCCC

+200  CTCCGACAGCAACACCCG  GCTGTGCGCGCGGTCCGCG  CGAGAGGGGCGCGGAGGGG  TGTGCGGAGAGCCAGCGCG  CGGGCCGCTGTACGTGGGC

+300  GCCGCGCCAGCCGGGTGAG  GAGGCTGGGTGCCTCGTGA  TGGGGCGGGGCTCCACTC  TCATCCGAGGCTTTGGCAG  GGCACAGGAGCCGCGGTGG

+400  TACCCGGGCTCCAGTTTGA  GAGGCCAGTTGTGGGTCTGG  GAGCGCAGGAGCCCGAGG  GCCTCAGCGGAAGTCATCAG  ACCATCAGGCTCACCGGC

+500  TGGGTGGCGGGAAGCCTTC  TCCTTGGACTACGGCTGCAA  GGCTGAACTGCCCTGGGCA  AGTGCTTAGGGACCAGAGG  ATCTATCAAACCTTCAGCT

+600  GGCTCAGCCAGGTTACAAG  TGTTCCAGCAATGGCGAGA  GGCTTCTCTGTCCCGGATC  CGCACCTAGTTAGGAGTGGT  ACTTAGGTTCGGATCTTTGC

+700  TGAAAGGATCCGGTTGGCAG  GGGATCGGTTTGCTCAGCC  CTGCTAGGCTGAGCACTAA  GTAGTCGGGCTGTCTCTC  TGGGTCTTACCATGCCTGG

+800  CGTTCTGGCTGGCCTGCAG  GGCAGTCAGTCTCACCCCTC  ATCATGAAAGGAAGCGTTG  GCTTCCCGTGGCTTGAGGT  GAGAGTGTCCAGACCCCTGC

+900  TTCTTGAAGCCCCACTCTG  TTTGCAAGTTTCTTATGATT  GGGATGGAGCAGCCAGTAGG  CAACTGGAGATCATATGGTC  CCAGGTAACTAGCAAGGCAT

+1000  GGTAGGGTCAGGCCAGGTCA  TGTGTCTGGAAGGATTAA  AACCTCAGAAGTAGCTATCC  CGTGCAAGGGAACCCGAGCA  CCCGGGCTCATCATTGATT

+1100  ATAGCTGGGTCTGCTAGTT  TCCACCACTGGGGGCTTTC  TGAGCCCTTGTCTCAGAA  AGTCAGGCGCTGGCTGTGAG  GTGTGCTACCTGTGGATTCA

+1200  GGTGGCTGTAGCTGGGAATG  ACTCTATGAGGAGGCCAC  CAGGCTACCTACCTTCCCC  ACAGAGAAGAGCCATG...
Met..

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Fig. 5. Nucleotide sequence of the rat fatty acid synthase gene from -1592 to +1276

The first exon containing 77 bases of untranslated sequence is boxed; an asterisk marks the start of the small percentage of transcripts which begin at -5. The 5'-end of the second exon (boxed) begins at +1265 and contains nine bases of untranslated sequence followed by the translation initiation codon. The putative Sp1 sites and sequences similar to the glucocorticoid, oestrogen/thyroid hormone and progesterone response elements and to FSE-1 are underlined (see the text).

Location of the transcription initiation site was confirmed by S1 nuclease protection experiments. A 191-nucleotide-long single-stranded genomic DNA, complementary to the 5'-end and extending beyond the apparent transcription initiation site of the fatty acid synthase mRNA, was hybridized to RNA from both liver and mammary gland and treated with S1 nuclease. The 3'-end of the DNA fragment was protected from digestion up to nucleotide 73 (Fig. 3c). A small proportion of the DNA probe was protected to a point five nucleotides further upstream. Thus the S1 nuclease and primer extension experiments indicate that the 5'-end of the mature fatty acid synthase mRNA is 87 nucleotides upstream from the translation initiation site.

Northern blots of RNA from various rat tissues were probed to confirm that the 5'-end of pFAS 27 did not arise from an alternatively spliced fatty acid synthase transcript occurring at low frequency. The insert from pFAS 27 hybridized to the characteristic doublet of 8.3 kb and 9.1 kb for fatty acid synthase in mRNAs derived from brain, lung, mammary gland and liver; the level of fatty acid synthase mRNA in mammary gland was considerably lower in pregnant compared with lactating rats, and fatty acid synthase mRNA was not detected in spleen (Fig.

4a). Significantly, the pFAS 27 probe also hybridized to a small RNA species (~800 nucleotides long) present only in lactating rat mammary gland. In contrast, a probe complementary to part of the sequence obtained directly from the fatty acid synthase mRNA recognized only the fatty acid synthase mRNA species (Fig. 4c); a probe complementary to a sequence at the 5'-end of pFAS 27 recognized only the small mRNA species (Fig. 4b). Thus the first 45 nucleotides of pFAS 27 appear to have been reverse-transcribed from a small mRNA, unrelated to fatty acid synthase, which is abundantly expressed in mammary gland during lactation. During construction of the cDNA library, this 45-nucleotide-long transcript was presumably spliced to an authentic fatty acid synthase cDNA which fortuitously lacked the nucleotides corresponding to the 5'-end of the mRNA.

Promoter sequences

Part of the rat fatty acid synthase genomic sequence (from the *SacI* site at -1592 to the translation initiation codon at +1276) is presented in Fig. 5. The region upstream of the major transcription initiation site (+1) contains sequences known to be part of promoters in other eukaryotic genes. An inverted CAAT

sequence (ATTGCC) is located in the rat fatty acid synthase gene at nucleotides -98 to -92 (see Fig. 5). The region from -290 to the cap site is very GC-rich (78 % G + C) and contains five sequence elements which conform to the Sp1 transcription factor binding site consensus sequence (G/T)(G/A)GGCG-(G/T)(G/A)(G/A)C/T [18] in nine out of ten positions.

The region from -33 to -26 contains a sequence similar to the TATA box found in approximately the same position in most genes [19]. However, the rat fatty acid synthase sequence contains a T in the second position, a relatively rare variation of the promoter element [20,21].

Many of the hormones which increase or decrease fatty acid synthase activity in mammals have been shown to interact with hormone response elements of other genes, thereby altering the rate of transcription. A search of the fatty acid synthase gene has revealed a number of sequences similar to known elements. The sequence starting at position -624 (Fig. 5) matches the oestrogen response element consensus sequence GGTCANNNTG-(A/T)CC [22] in nine out of ten positions, as well as the thyroid hormone response element for rat growth hormone, GGGAC·GTGACCG [23], in nine out of 12 positions. Thyroid hormone has been implicated in the regulation of fatty acid synthase expression since, when administered to diabetic rats, it restores fatty acid synthase enzyme levels [24].

Other possible steroid hormone response elements are located within the first intron. The rat fatty acid synthase sequence starting at +611 (GGTTACAAGGTGTTCTG) matches the mouse mammary gland tumour virus glucocorticoid response element in 13 out of 16 positions (underlined) and the glucocorticoid response element consensus sequence GTTACANNNTGTTCT [22] in ten out of 12 positions, and thus may play a role in dexamethasone-induced gene activation. Also, progesterone has been shown to increase the transcription rate of the fatty acid synthase gene in human breast cancer cells [25], though its effect on fatty acid synthase levels in normal mammary gland cells appears to be inhibitory [26]. Response elements for the glucocorticoid and progesterone receptors appear to be functionally indistinguishable [27]. However, the rat fatty acid synthase sequence starting at +908, AGCCCACTTCTGTTTGCA, matches the progesterone response element consensus, ATC(C/T)(C/T)ATT(A/T)TCTG(G/T)TTGTA [28], in 14 out of 19 positions (underlined), and thus may serve as a progesterone element for this gene.

Several studies have suggested that cyclic AMP regulates fatty acid synthase gene expression, positively in human fetal lung [29] and negatively in mouse liver [30]. Transcriptional regulation of gene expression by cyclic AMP is thought to involve either a cyclic AMP response element or AP-2 elements [31]. There are eight sequences in the GC-rich region between nucleotides -475 and -25 which match these AP-2 sites in seven out of eight positions, but none which appears to match the cyclic AMP response element (results not shown). Thus it is possible that cyclic AMP control of fatty acid synthase gene expression may be mediated through AP-2 sites in the 5'-flanking region.

Sequences common to the 5'-flanking regions of several genes whose transcription increases during adipocyte differentiation have been termed fat-specific elements (FSEs) [32]. The 13-base sequence starting at nucleotide +1005 of the rat fatty acid synthase gene (GGGTCAGGCCAGG) matches the FSE-1 consensus [GGC(T/A)CTGGTCA(G/T)G] in ten positions (underlined). Since fatty acid synthase synthesis is induced in differentiating adipocytes [33], this FSE-1-like element may play a role in mediating fatty acid synthesis gene induction in adipose cells.

The transcription initiation sites and the TATA and CAAT

boxes can be readily identified for the rat fatty acid synthase gene from the data presented here. However, identification of candidates for Sp1, AP-2 and FSE-1 sites and the various hormone response elements for this gene is tentative at this time. Direct experimental evidence for the location of these regulatory sites is required.

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