

Expression of GATA-binding transcription factors in rat hepatocytes

Koichi Matsuda, Yoshiko Kobune, Chiseko Noda*, Akira Ichihara¹

Institute for Enzyme Research, University of Tokushima, Kuramoto-cho, Tokushima 770, Japan

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Abstract Recently, we demonstrated that a DNA-binding protein(s) is involved in transcriptional repression of the rat serine dehydratase gene in fetal liver. Here, we report that a GAT(A/T) motif is the target sequence for the DNA-binding protein. By screening a fetal liver cDNA library, we isolated a rat homolog of GATA-1. Rat GATA-1 expressed as a GST-fusion protein in *E. coli* bound to the GAT(A/T) motif in the serine dehydratase gene. Northern analysis show that GATA-1 and GATA-4 mRNAs are expressed in fetal hepatocytes.

Key words: DNA-binding protein; GATA-family; GATA-4; Rat hepatocyte

1. Introduction

Parenchymal hepatocytes that constitute about 90% of the cell mass in the liver of adult rats have a variety of specific functions. Serine dehydratase (SDH), which catalyzes conversion of serine to pyruvate and ammonia, is expressed predominantly in hepatocytes of the liver. During development of rats, its expression is repressed in fetal liver and turned on just after birth [1]. Recently, we found that a negative-acting transcription factor(s) is involved in the repression of the SDH gene. This factor(s) binds to regions B and I of the SDH gene, and possibly recognizes sequences homologous to the binding site of GATA-binding transcription factors [2].

Members of the GATA-binding transcription factor family all have a highly conserved protein-domain, which is necessary for DNA-recognition and consists of two zinc fingers, and they bind to specific DNA sequences with the GATA-motif. In vertebrates, five GATA-binding proteins have been identified [3]. GATA-1 is found only in cells of myeloid lineage (erythroid cells, mast cells, and megakaryocytes) [4–8], with the exception of an abundant testis-specific form transcribed from an alternative promoter [9]. In mammals, the liver participates in erythropoiesis during the fetal period. Thus, GATA-1 is found in fetal liver, but not in adult liver [8,10]. GATA-2 is expressed in a wide variety of tissues [8,11–13] and GATA-3 is most abundantly expressed in T lymphocytes and in the developing central nervous system [8,14,15]. GATA-4 which is identical to GATA-GT2 is highly expressed in gastric mucosa, and its mRNA signal is also detected with RNAs from testes, ovary, intestine, and heart [16–18]. The fifth GATA-factor, GATA-GT1, was found as a transcription factor for the H⁺/K⁺-ATPase α and β genes in stomach, as well as GATA-GT2 [18].

In this paper we report that a DNA-binding protein(s) in fetal rat liver recognizes a GAT(A/T) motif of regions B and I of the SDH gene, and that fetal rat hepatocytes express GATA-1 and -4 mRNAs.

2. Materials and methods

2.1. Animals and isolation of hepatocytes

Fetal rats on day 18–20 of gestation, neonatal rats on day 1, and 6 to 8-week-old male rats of the Wistar strain weighing about 200 g were used. Hepatocytes were isolated as described previously [2].

2.2. Gel mobility shift assay

Preparation of fetal liver nuclear extract and gel mobility shift assay were carried out as described previously [2]. The probes used were 5' end-labeled oligonucleotides corresponding to the sequences of regions B and I of the SDH gene [2] and the GATA-1 binding site of the mouse α -globin promoter (M α P) [5].

2.3. Amplification of zinc finger domains by the reverse transcriptase (RT)-polymerase chain reaction (PCR)

Total RNAs of various tissues and hepatocytes were isolated by the guanidine thiocyanate procedure [19] and poly(A)⁺ RNA was purified by oligo(dT)₃₀-latex (Oligotex-dT30, Takara Shuzo). For RT-PCR, synthesis of first stranded cDNAs was performed with a First-Strand cDNA Synthesis kit (Pharmacia) with poly(A)⁺ RNAs from liver tissues and hepatocytes as templates. Primers were designed to generate a 270 bp PCR product corresponding to the conserved regions of two zinc fingers: upstream primer, 5'-GA(A/G)G(C/G) CAG(A/G)GAGTGT-GTGAACCTG(C/T)GG(A/G)GCA-3'; downstream primer, 5'-GTGN-AGCTTGTAGTAGAG(C/G)CG(A/G)CAGGCATT-3'. Amplification was carried out for 5 min at 94°C followed by 30 cycles at 94°C for 1 min, 65°C for 2 min, and 72°C for 2 min with a final 4 min extension at 72°C.

2.4. Northern blot analysis

Samples of 5 μ g of Poly(A)⁺ RNA from various tissues and isolated hepatocytes were electrophoresed in 1% agarose gel containing 1.5% formaldehyde, and transferred to a Hybond-N⁺ nylon membrane (Amersham). DNA fragments of rat GATA-1 complementary DNA (cDNA, nucleotides 1,108–1,674) reported in this paper (Fig. 2) and mouse GATA-4 cDNA (nucleotides 1,058–1,385), which had been synthesized by PCR on the basis of the sequence reported by Arceci et al. [16] and generously provided by Dr. Yamamoto (Tohoku University), were labeled with [α -³²P]CTP using a commercial kit for multiprimer DNA labeling (Takara Shuzo). Hybridization with these DNA fragments as probes was carried out as described [1].

2.5. Cloning of cDNA for rat GATA-1 and its bacterial expression

A cDNA library from the liver of fetal rat on day 18 of gestation was constructed in the *Not*I and *Xho*I sites of λ ZAPII vector (Stratagene). About 4×10^5 plaques were screened by hybridization with a DNA fragment(s) that had been synthesized by RT-PCR with fetal liver poly(A)⁺ RNA as described above (section 2.3), purified and 5' end-labeled. After plaque hybridization, the pBluescript plasmid was excised and examined by DNA sequence analysis using an AutoRead Sequencing kit (Pharmacia).

For construction of a glutathione S-transferase(GST)-GATA-1

*Corresponding author. Fax: (81) (886) 33 4223.

¹Present address: Department of Environmental Science, Tokushima Bunri University, Tokushima 770, Japan.

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fusion gene, the coding region of the rat GATA-1 cDNA (nucleotides 107 to 1615 in Fig. 2) was introduced into the *Eco*RI site of the bacterial expression vector pGEX-3X (Pharmacia). The GST-GATA-1 fusion protein was expressed and purified essentially as described by Smith and Johnson [20]. Briefly, *E. coli* HB101 cells transformed with GST-GATA-1 were grown to A_{600} 1.0, and IPTG was added at a final concentration of 0.5 mM. After further incubation for 3.5 h the cells were pelleted, washed with phosphate-buffered saline (PBS), and stored at -80°C . The cells were suspended in 4 volumes of PBS containing 1% Triton X-100, lysed by sonication, and centrifuged at $28,000 \times g$ for 15 min. The clear supernatant was applied to a glutathione-Sepharose 4B (Pharmacia) column. The column was washed with PBS, and then the GST-GATA-1 fusion protein was eluted with 50 mM Tris-HCl buffer, pH 8.0, containing 5 mM glutathione.

3. Results and discussion

3.1. RT-PCR analysis of GATA-factor related protein transcripts

To examine expression of a member(s) of the GATA-binding transcription factor family in hepatocytes, we first performed RT-PCR analysis. Primers were designed to generate a DNA fragment corresponding to the conserved zinc finger domains of GATA-binding proteins. As expected, a 270 bp PCR product(s) was detected with the poly(A)⁺ RNA fraction from fetal hepatocytes by RT-PCR analysis (Fig. 1). The amount of the PCR product(s) with the RNA fraction from fetal hepatocytes was more than those with fractions from neonatal liver, adult liver, and adult hepatocytes.

3.2. Isolation of a cDNA clone encoding rat GATA-1

Fetal liver is known to function as a hematopoietic organ. Hence, GATA-1 is present in fetal liver owing to high expression of this factor in erythroid cells [8,10]. To determine whether hepatocytes in fetal liver express a known or new member of the GATA-binding protein family, we had tried to isolate cDNA clones from fetal liver encoding GATA-binding proteins including GATA-1. A liver cDNA library of rat fetuses on day 18 of gestation in λ phage was screened with a labeled RT-PCR product(s) corresponding to the zinc finger domains as a probe. One cDNA clone of 1,863 nucleotides hybridizing with this probe(s) was obtained by screening approximately

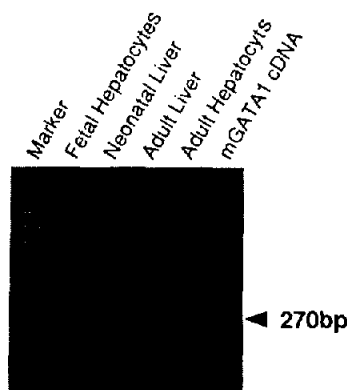


Fig. 1. Expression of a member of the GATA-binding factor family in rat liver and hepatocytes. RT-PCR products were analyzed in 2% agarose gel. Mouse GATA-1 (mGATA-1) cDNA was used as a positive control for amplification by the PCR. DNA molecular size markers were 72 to 1,353 bp (Nippon Gene). The arrowhead indicates the position of the expected PCR product of 270 bp.

1	GTGCTCAAGCCAAAGCCAGCGAGGACTCCCTTGGGATCATATGACCTCACCACACC
61	ACTAAGCTGCTGAACCTTCTGCATCAACAGCCAGGTTAAACCCAGTGTCCCATGG
121	ATTTTCCTCGTCTAGGGGCGCTGGGAACCTCAGAACCTTGGCCCATTTTGGGATCTG
181	CCCTGGTCTCTCGACATCAGACTCAGCGGGTTCTTCTTCTTGGCCGAGAGCTTGG
241	ATACAGGATCTTCTTCCACTTCTCCAAATGCGCCAGGCGAGCCGACGCGACTGGCT
301	ACTACAGAGAGCTGAGGGCTTACAGACACTCCCACTTCTTACAGGTGTACCCGCTGCTCA
361	ACAGTATGGAGGGAATCCGAGGAGCTCACCCTATGCTAGCTGGGCTATAGCAAGACGG
421	CGCTTACCTTGCCTCAACTGTATGTCCTCAGTCTAGGATGCCCTTCCGACACCTGG
481	AAGACCCAGATGGGAAGAACAAATACATCTTGGAAACCTTGAAGACGAGCGGCTGA
541	GTCCAGACTCTCTGACCTGGGGAGCTGACCTGCTTACATCTCTTCCCTACGACAGTG
601	CTTATGGGGAGCTGACCTTCCAGTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTT
661	CAGGACCTATTCTTCCCCCAAGTTTCATGGAAGCTTACCTTGGCTCTTCTTGGGCGCA
721	ATGCTTCTTGAATCTGCGCAAGCGTACTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTT
781	ATGCTTCTTGAATCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTT
841	ATGCTTCTTGAATCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTT
901	ATGCTTCTTGAATCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTT
961	ATGCTTCTTGAATCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTT
1021	ATGCTTCTTGAATCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTT
1081	ATGCTTCTTGAATCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTT
1141	ATGCTTCTTGAATCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTT
1201	ATGCTTCTTGAATCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTT
1261	ATGCTTCTTGAATCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTT
1321	ATGCTTCTTGAATCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTT
1381	ATGCTTCTTGAATCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTT
1441	ATGCTTCTTGAATCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTT
1501	ATGCTTCTTGAATCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTT
1561	ATGCTTCTTGAATCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTT
1621	ATGCTTCTTGAATCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTT
1681	ATGCTTCTTGAATCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTT
1741	ATGCTTCTTGAATCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTT
1801	ATGCTTCTTGAATCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTT
1861	ATGCTTCTTGAATCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTT

Fig. 2. Nucleotide and predicted amino acid sequence of rat GATA-1. The zinc finger domains are shadowed. The termination codon is indicated by an asterisk.

4×10^5 recombinant phage plaques. DNA sequencing revealed that this clone contained a long open reading frame encoding a protein of 413 amino acids with a molecular mass of 42.9 kDa (Fig. 2). The predicted amino acid sequence of this cDNA had 94% overall identity with mouse GATA-1 [5] and their zinc finger domains (200–289) showed >98% identity (data not shown). Thus, we concluded that this cDNA was a rat homolog of GATA-1. We failed to identify any other cDNA clones of GATA-binding proteins.

3.3. Comparison of the DNA binding specificity of the fetal liver nuclear protein(s) and that of GATA-1

Recently, we found that regions B and I of the SDH gene contain a GATA and GATT sequence, respectively, in their lower strands (Fig. 3d). Competition studies suggested that DNA-binding sites in these regions are recognized by the same protein or similar proteins [2]. To determine whether the GATA and GATT sequences in these regions are essential for binding of this protein and whether this protein is the same as GATA-1, we examined their DNA-binding specificities by gel mobility shift assay. For these experiments, point mutations were introduced into the GATA and GATT motifs. As reported previously [2], Fig. 3a shows that a DNA-binding protein(s) present in fetal liver interacted with region B (lanes 1–4). A signal with higher mobility was considered to be non-specific,

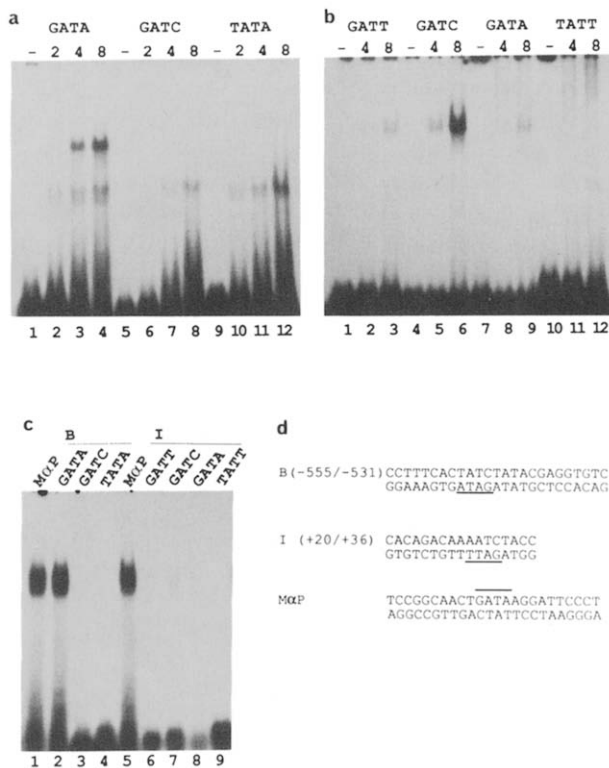


Fig. 3. Comparison of DNA-binding specificities of the nuclear protein(s) and recombinant rat GATA-1. (a) Nuclear extract (2, 4 or 8 μg protein) from fetal rat liver was examined by gel mobility shift assay for binding to ³²P-labeled oligonucleotide of region B (lanes 1–4) and for a point mutation in the GATA motif (lanes 5–12, as indicated). Lanes for each probe only are indicated by a minus sign. (b) Binding to region I (lanes 1–4) and three mutated probes (lanes 4–12, as indicated). Other explanations are as for Fig. 3a. (c) For comparison, rat GATA-1 protein was expressed as a GST fusion protein. The probes used were as for Figs. 3a and Fig. 3b, except for the GATA-1 binding site of mouse α-globin promoter (MαP, lanes 1 and 5). Samples of 3 μg of the fusion protein were used for the binding. (d) Nucleotide sequences of regions B and I, and MαP.

because it was not eliminated by the addition of excess unlabeled oligonucleotide of region B to the reaction mixture. Two mutation probes, which had the sequences GATC and TATA, respectively (lanes 5–8 and lanes 9–12, respectively), instead of the GATA-motif, did not form a DNA–protein complex(s).

On binding to region I, a GATT to GATA-mutation probe (Fig. 3b, lanes 7–9) interacted with the DNA-binding protein(s) as strongly as the native region I (lanes 1–3), but a GATT to TATT-mutation probe did not (lanes 10–12). Unexpectedly, the DNA-binding protein(s) had a stronger preference for a GATC-mutation probe (lanes 4–6) than for the native region I. The formation of this DNA–protein complex(s) was eliminated by the presence of unlabeled oligonucleotide corresponding to region B or I (data not shown). Therefore, it is unlikely that different DNA-binding proteins bind to the native region I and GATC-mutation probes. This observation is in contrast with the loss of binding activity of a similar GATC-mutation probe in region B (Fig. 3a, lanes 5–8). On the basis of recent reports [21–23], GATA-1, -2 and -3 recognize the GAT(A/T) motif and also the core motif (T/C)AAG. Therefore, the recognition of the GATT to GATC-mutation probe in region I is

explained as follows: this point mutation generates a CAAG sequence in the middle of its upper strand (CAAA→CAAG, see Fig. 3d) and the CAAG sequence may function as a target sequence for the DNA-binding protein(s). In contrast, the GATA to GATC-mutation in region B does not generate a CAAG sequence. Alternatively, interaction of DNA with the binding protein(s) is influenced by sequences surrounding the GAT(A/T) motif as described previously [22,23]. These results of gel mobility shift assays show that target DNA sequences for the DNA-binding protein(s) present in fetal rat liver is similar to those for GATA-binding transcription factors.

To compare the DNA-binding specificity of the protein(s) present in the fetal liver with that of GATA-1, GATA-1 protein was expressed in *E. coli* as a GST fusion protein. The GST-GATA-1 fusion protein was shown to bind specifically to the GATA-1 binding site of the mouse α-globin promoter (MαP) (Fig. 3c, lanes 1 and 5), while the full-length GST protein alone did not (data not shown). The fusion protein interacted with region B (lane 2), as strongly as with MαP, but did not have preference for the GATC- and TATA-mutation probes (lanes 3 and 4, respectively). Although GST-GATA-1 showed very weak binding to region I and its mutation probes (lanes 6–9), we should be considerate that this GATA-1 protein is the fusion protein expressed in *E. coli*. Thus, these results suggest that the binding specificity of the DNA-binding protein(s) in fetal liver is similar to that of GATA-1.

3.4. Expression of GATA-4 and GATA-1 in hepatocytes

GATA-4/GATA-GT2, in contrast to GATA-1, -2 and -3, is not found in cells of hematopoietic lineage. Its mRNA was detected in the stomach, testes, intestine, and ovary, and very

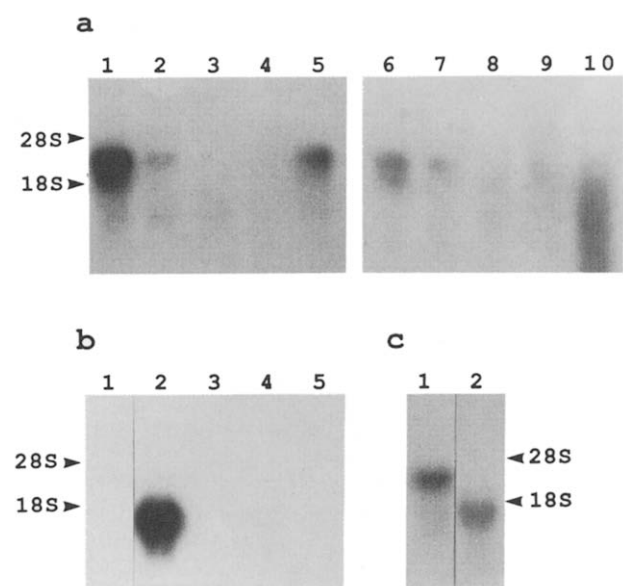


Fig. 4. Northern blot analysis of GATA-4 and GATA-1 mRNA levels in various tissues and isolated hepatocytes. (a) Samples of 5 μg poly(A)⁺ RNA from fetal (lanes 1–5) and adult (lanes 6–10) tissues of rats were subjected for Northern blot analysis with the GATA-4 probe. Lanes 1 and 6, heart; lanes 2 and 7, liver; lanes 3 and 8, kidney; lanes 4 and 9, lung; lanes 5 and 10, small intestine. (b) Northern blot analysis of GATA-1 mRNA in fetal rat tissues. Other conditions were as for Fig. 4a. (c) Poly(A)⁺ RNA (5 μg/lane) from isolated hepatocytes of fetal rats was subjected for Northern blot analysis with the GATA-4 probe (lane 1) and GATA-1 probe (lane 2).

slightly in the heart, lung, and liver [16–18]. Thus, we examined the expression of GATA-4 in various tissues from fetal and adult rats, and in isolated hepatocytes from fetal liver. In various fetal tissues tested, a 4 kb mRNA that was similar in size to GATA-4 mRNA was detected with poly(A)⁺ RNA from heart and small intestine, and slightly with that from liver (Fig. 4a). In adult rats, GATA-4 mRNA signal was found with poly(A)⁺ RNA from the heart and small intestine, with very weak signals in the liver and lung. The smear of the signal for intestine probably resulted from degradation of RNA during its isolation. The mRNA levels in the heart and liver, respectively, were much lower and slightly lower than those in fetal rats.

Fig. 4b shows that expression of GATA-1 mRNA (1.9 kb) was specific to fetal liver, consistent with previous results [8,10]. To exclude hematopoietic cells from liver tissues of fetuses, we isolated the hepatocytes and subjected poly(A)⁺ RNA from these cells to Northern blot analysis. Fig. 4c shows the presence of GATA-4 and -1 mRNAs in fetal hepatocytes, although GATA-1 signal in hepatocytes might be overestimated because of some contamination with erythroid cells.

The present report provides the first evidence that parenchymal hepatocytes in fetal liver express members of GATA-binding transcription factor family. The nuclear protein(s) present in fetal liver binds to the GAT(A/T) motif in the SDH gene is possibly either GATA-1 or -4, or both. On the basis of our previous finding, GATA-1 and/or GATA-4 should function as negative trans-acting factors of the SDH promoter. GATA-binding proteins have been shown to have effects on transcription by binding to target sequences and so may have important roles in development in specific tissues or cells. In fact, GATA-1 has been shown to be essential for development of erythroid cells [3]. Thus we suppose that GATA-1 and -4 have a critical role in development of hepatocytes.

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