

**ISOLATION AND SEQUENCING OF RAT LIVER BILIRUBIN
UDP-GLUCURONOSYLTRANSFERASE cDNA: POSSIBLE ALTERNATE
SPLICING OF A COMMON PRIMARY TRANSCRIPT**

Hiroshi Sato^{1*}, Osamu Koiwai², Kazushi Tanabe³ and Shigeo Kashiwamata¹

Departments of ¹Perinatology and ²Biochemistry, Institute for Developmental
Research, Aichi Prefecture Colony, Kasugai Aichi 480-03, Japan

³Biophysics Unit, Aichi Cancer Center Research Institute,
Chikusa-ku, Nagoya 464, Japan

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SUMMARY: A 1763-bp cDNA for rat liver bilirubin UDP-glucuronosyl-transferase (UDPGT) was isolated. Bilirubin UDPGT activity was demonstrated by transfection of the pcDL1 vector carrying the cDNA into COS7 monkey kidney cells. The cDNA shares an identical 913-bp sequence (corresponding to the C-terminal 247 amino acid residues) with that for rat liver 3-methylcholanthrene-inducible 4-nitrophenol UDPGT including the locus where a -1 frameshift mutation was found in the 4-nitrophenol UDPGT cDNA from the jaundiced homozygous Gunn rat. The result suggests that both the UDPGTs are derived from a common primary-transcript and that the multiple defects of UDPGT isoenzymes observed in the homozygous Gunn rat may be produced by a single-mutated-locus after an alternative splicing of the 5' end region. © 1990

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In 1938, Gunn described a mutant strain of Wistar rats (Gunn rat) that showed hereditary hyperbilirubinemia (1). The homozygous Gunn rat lacks hepatic UDP-glucuronosyltransferase (UDPGT, EC 2.4.1.17) activity towards bilirubin (2) and has been used as an animal model for human Crigler-Najjar syndrome type I (3,4). It has been also reported that the Gunn rat has genetic deficiencies of both hepatic bilirubin (Bil) and 3-methylcholanthrene (3-MC)-inducible 4-nitrophenol (4-NP) UDPGT activities (5). Recently, Iyanagi et al. (6) further clarified the genetic defect of 4-NP UDPGT in the homozygous Gunn rat liver as a -1 frameshift mutation. They also suggested that in the liver of 3-MC-untreated homozygotes, there existed another mRNA that had the same defective sequence

*To whom correspondence should be addressed.

²Present address: Department of Biochemistry, Aichi Cancer Center Research Institute, Chikusa-ku, Nagoya 464, Japan.

as proven in the mutated 4-NP UDPGT. On the other hand, Nagai et al. (7) have demonstrated by the cross-breeding of mutant rat strains that Bil UDPGT is located on the same chromosome as 4-NP UDPGT. Based on these findings, we tried to isolate Bil UDPGT cDNA from a rat liver cDNA library using a synthetic 40-mer oligonucleotide probe that has a complementary sequence with the 4-NP UDPGT cDNA.

MATERIALS AND METHODS

Screening of cDNA library. A cDNA library from the rat liver was obtained from Clontech Lab., Inc., Palo Alto, CA. We isolated a cDNA for Bil UDPGT by screening the library with a synthetic probe covering the nucleotide sequence from positions 1,520 to 1,559 in the rat liver 3-MC-inducible 4-NP UDPGT cDNA (8). Detailed procedures for the screening of cDNAs were performed essentially by the method of Maniatis et al. (9,10).

Transfection of Bil UDPGT cDNA clone. The cDNA isolated was subcloned into the pcDL1 vector (a generous gift from Dr. T. Yokota). The cloning site in the vector has been converted from the EcoR I site to the Xba I site in our laboratory. The incubation mixture for the polymerase chain reaction (PCR) contained in a total volume of 100 μ l of 60 mM Tris-HCl buffer, pH 8.8, 1 μ g of cloned λ gt 11 DNA, 1 μ M primers, 1.5 mM dNTPs, 10 % dimethylsulfoxide, 10 mM MgCl₂, 10 mM (NH₄)₂SO₄ and 200 μ g/ml gelatin. Transfection of the cDNA (20 μ g) into monkey kidney COS7 cells (4 x 10⁶) was performed as described by Koizumi et al. (10). Transfected cells were incubated for 72 h without treatment of chloroquine. Bil UDPGT activity was detected by thin layer chromatography according to Jackson et al. (11). The bilirubin concentration in the assay mixture was 0.5 mM. [Glucuronosyl-U-¹⁴C]UDP-glucuronic acid was purchased from ICN Biochem., Inc., Irvine, CA. p-Nitrophenyl- β -D-glucuronide was obtained from Sigma Chemical Co., St. Louis, MO.

RESULTS

Isolation of rat Bil UDPGT cDNA. Plaque screening of the rat liver λ gt 11 cDNA library (1 x 10⁵ independent clones) with the radiolabeled oligonucleotide probe identified four UDPGT cDNA clones. The entire nucleotide sequences of these clones were determined by the M13 dideoxy chain termination method (12). The results obtained clearly showed that the isolated clones were a new type of UDPGT cDNA. The longest clone (pSK1) contained 1,763-bp with an open reading frame of 1,593-bp. Figure 1 shows the nucleotide sequence together with its predicted amino acid sequence of the pSK1 clone. Comparison of the sequence of the clone with that of the 4-NP UDPGT cDNA revealed that both the cDNAs shared an identical sequence of 913-bp (C-terminal 247 amino acid residues from positions 285 to 531). However, there is a considerable divergence of the amino acid sequence in the N-terminal region between them.

Expression of the rat Bil UDPGT cDNA clone in COS7 cells. To confirm that this new clone was for Bil UDPGT, we examined the expression of pSK1 in mammalian cells. A cDNA fragment of 1,624-bp including an extra two bases (AT) at the 5' end of the clone was synthesized by PCR. Synthetic primers containing the Xba I site (5'AGATCTAGATGGGATTGTGTGCACCCCTTCG3' and AGATCTAGAATA

(AT)G GGA TTG TGT GCA CCC CTT CGA GGA CTC TCA GGA CTG CTG CTC CTG CTG TGT GCC CTG CCC TGG GCT GAA GGT GGA AAG GTG CTG GTG (Met) Gly Leu Cys Ala Pro Leu Arg Gly Leu Ser Gly Leu Leu Leu Leu Cys Ala Leu Pro Trp Ala Gly Gly Lys Val Leu Val	90
TTT CCC ATG GAG GGC AGC CAC TGG CTG AGC ATG AGG GAT GTC GTG AGG GAG CTC CAC GCC CGA GGT CAC CAG GCT GTG GTC CTG GCT CCA Phe Pro Met Glu Gly Ser His Trp Leu Ser Met Arg Asp Val Val Arg Glu Leu His Ala Arg Gly His Gln Ala Val Val Leu Ala Pro	180
GAG GTG ACT GTG CAC ATG AAA GGA GAG GAC TTC TTC ACC CTC CAA ACC TAT GCC TTT CCA TAT ACC AAG GAA GAA TAT CAG CGG GAA ATA Glu Val Thr Val His Met Lys Gly Glu Asp Phe Phe Thr Leu Gln Thr Tyr Ala Phe Pro Tyr Thr Lys Glu Glu Tyr Gln Arg Glu Ile	270
CTG GGC AAC GCT AAG AAG GGC TTC GAA CCA CAA CAT TTT GTG AAG ACT TTC TTT GAA ACT ATG GCA TCT ATA AAA AAG TTT TTC GAT CTC Leu Gly Asn Ala Lys Lys Gly Phe Glu Pro Gln His Phe Val Lys Thr Phe Phe Glu Thr Met Ala Ser Ile Lys Lys Phe Phe Asp Leu	360
TAC GCA AAT TCT TGT GCA GCT CTA TTG CAC AAT AAG ACC CTG ATC CAG CAA CTG AAT TCC AGT TCC TTC GAT GTG GTC TTA ACA GAC CCT Tyr Ala Asn Ser Cys Ala Ala Leu Leu His Asn Lys Thr Leu Ile Gln Gln Leu Asn Ser Ser Ser Phe Asp Val Val Leu Thr Asp	450
GTT TTC CCC TGT GGA GCA TTA CTG GCC AAG TAT CTA CAG ATT CCT GCT GTG TTT TTT CTG CGC TCT GTT CCC TGT GGC ATA GAC TAT GAG Val Phe Pro Cys Gly Ala Leu Leu Ala Lys Tyr Leu Gln Ile Pro Ala Val Phe Phe Leu Arg Ser Val Pro Cys Gly Ile Asp Tyr Glu	540
GCT ACA CAA TGT CCG AAA CCT TCC TCT TAT ATT CCG AAC CTA CTC ACA ATG CTT TCT GAC CAC ATG ACC TTC CTG CAA AGG GTC AAG AAC Ala Thr Gln Cys Pro Lys Pro Ser Ser Tyr Ile Pro Asn Leu Leu Thr Met Leu Ser Asp His Met Thr Phe Leu Gln Arg Val Lys Asn	630
ATG CTG TAC CCT CTG ACC TTG AAG TAC ATT TGC CAT TTA TCA ATC ACT CCC TAT GAA AGC CTG GCC TCT GAG CTT TTG CAG AGA GAA ATG Met Leu Tyr Pro Leu Thr Leu Lys Tyr Ile Cys His Leu Ser Ile Thr Pro Tyr Glu Ser Leu Ala Ser Glu Leu Leu Gln Arg Glu Met	720
TCT TTA GTG GAG GTT CTC AGT CAT GCA TCT GTG TGG CTG TTC CGA GGG GAC TTT GTG TTT GAC TAC CCG AGG CCC ATC ATG CCT AAT ATG Ser Leu Val Glu Val Leu Ser His Ala Ser Val Trp Leu Phe Arg Gly Asp Phe Val Phe Asp Tyr Pro Arg Pro Ile Met Pro Asn Met	810
GTC TTC ATT GGA GGC ATA AAC TGT GTC ATC AAG AAG CCC CTC TCT CAG GAA TTT GAA GCC TAT GTC AAC GCC TCC GGA GAA CAT GGC ATC Val Phe Ile Gly Gly Ile Asn Cys Val Ile Lys Lys Pro Leu Ser Gln Glu Phe Glu Ala Tyr Val Asn Ala Ser Gly Glu His Gly	900
GTG GTT TTC TCT TTG GGA TCC ATG GTC TCA GAG ATT CCA GAG AAG AAA GCG ATG GAA ATC GCT GAG GCT TTG GGC AGA ATT CCT CAG ACG Val Val Phe Ser Leu Gly Ser Met Val Ser Glu Ile Pro Glu Lys Lys Ala Met Glu Ile Ala Glu Ala Leu Gly Arg Ile Pro Gln Thr	990
CTC CTG TGG CGC TAC ACC GGA ACT AGA CCA TCG AAC CTT GCA AAG AAC ACT ATT CTT GTC AAA TGG CTA CCC CAA AAC GAT CTG CTT GGT Leu Leu Trp Arg Tyr Thr Gly Thr Arg Pro Ser Asn Leu Ala Lys Asn Thr Ile Leu Val Lys Trp Leu Pro Gln Asn Asp Leu Leu Gly	1080
CAT CCA AAG GCT CGG GCG TTC ATC ACA CAC TCC GGT TCC CAT GGT ATT TAT GAA GGA ATA TGC AAT GGG GTT CCA ATG GTG ATG ATG CCC His Pro Lys Ala Arg Ala Phe Ile Thr His Ser Gly Ser His Gly Ile Tyr Glu Gly Ile Cys Asn Gly Val Pro Met Val Met Met Pro	1170
TTG TTT GGT GAT CAG ATG GAC AAC GCC AAG CGC ATG GAA ACT CGG GGA GCT GGG GTG ACC CTG AAT GTC CTG GAA ATG ACT GCC GAT GAT Leu Phe Gly Asp Gln Met Asp Asn Ala Lys Arg Met Glu Thr Arg Gly Ala Gly Val Thr Leu Asn Val Leu Glu Met Thr Ala Asp Asp	1260
TTG GAA AAC GCC CTT AAA ACT GTC ATC AAT AAC AAG AGT TAC AAG GAG AAC ATC ATG CGC CTC TCC AGC CTT CAC AAG GAC CGT CCT ATC Leu Glu Asn Ala Leu Lys Thr Val Ile Asn Asn Lys Ser Tyr Lys Glu Asn Ile Met Arg Leu Ser Ser Leu His Lys Asp Arg Pro	1350
GAG CCT CTG GAC CTG GCT GTG TTC TGG GTG GAG TAC GTG ATG AGG CAC AAG GGG GCG CCA CAC CTG CGC CCC GCC GCC CAC GAC CTC ACC Glu Pro Leu Asp Leu Ala Val Phe Trp Val Glu Tyr Val Met Arg His Lys Gly Ala Pro His Leu Arg Pro Ala Ala His Asp Leu Thr	1440
TGG TAC CAG TAC CAC TCC TTG GAC GTG ATT GGC TTT CTC CTG GCC ATC GTG TTG ACG GTG GTC TTC ATT GTC TAT AAA AGT TGT GCC TAT Trp Tyr Gln Tyr His Ser Leu Asp Val Ile Gly Phe Leu Leu Ala Ile Val Leu Thr Val Val Phe Ile Val Tyr Lys Ser Cys Ala Tyr	1530
GGC TGC CGG AAA TGC TTT GGG GGA AAG GGT CGA GTG AAG AAA TCA CAC AAA TCC AAG ACC CAC TGA Gly Cys Arg Lys Cys Phe Gly Gly Lys Gly Arg Val Lys Lys Ser His Lys Ser Lys Thr His	1596
GAAGTGGCAGGAAGTGAAGGAGAAGTATTAGTCCCTTATCCAATCAGTTGAACTTGGAAACAAGTGTAAATCCATGTTGCTTTTATTAGGGAAATAATTACCATACACTATACCC CAGAACATTTTTTTCTTTCTTTCTTTTTCGGAGCTGGGGACCSAAACC	1715 1765

Fig. 1. Nucleotide sequence of bilirubin UDPGT cDNA and its predicted amino acid sequence. The nucleotides are numbered on the right-hand side. The nucleotide sequence identical with that of the 3-MC-inducible 4-NP UDPGT cDNA is indicated by a closed line. An arrowhead points to the locus where one base deletion occurs in the 4-NP UDPGT cDNA of the homozygous Gunn rat. Parentheses indicate the 5' end two nucleotides and N-terminal methionine that have been introduced by PCR.

CTTCTCCTTCACTTCC) were utilized for production of the cDNA fragment. The synthesized cDNA fragment was inserted into the Xba I site of the pcDL1 expression vector in the correct and opposite orientations, and the constructed

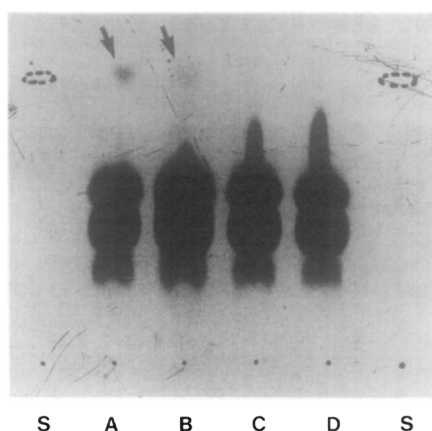


Fig. 2. Glucuronidation of bilirubin by Bil UDPGT expressed in COS7 cells - autoradiography of a thin layer chromatogram. A and B, transfected by pcDL1 containing the cDNA in the correct orientation (duplicate experiments); C, transfected by pcDL1 with the cDNA inserted in the opposite orientation; D, non-transfected COS7 cells; S, authentic 4-NP glucuronide (dotted circles). The reaction mixture (11) contained the homogenate of COS7 cells equivalent to 70 μ g protein and 9.25 kBq [glucuronosyl- U - 14 C]UDP-glucuronic acid. Arrows, enzymically formed bilirubin conjugate with UDP-[glucuronosyl- U - 14 C]glucuronic acid.

vectors were transfected into COS7 cells. Figure 2 shows Bil UDPGT activity detected by autoradiography on a thin layer chromatogram. Only the pcDL1 vector carrying the cDNA fragment in the correct orientation expressed the enzyme activity in COS7 cells. Gross estimation of the activity showed that Bil UDPGT expressed in COS7 cells was comparable to that in rat hepatic cells.

DISCUSSION

In this paper, we presented the nucleotide sequence of a cDNA clone encoding rat liver Bil UDPGT (Fig. 1). Although the isolated cDNA was lacking in two bases (AT) at the 5' end of the full length open reading frame that compared with the 4-NP UDPGT cDNA, the cDNA fragment synthesized by PCR to introduce the initiation codon at the 5' end successfully expressed Bil UDPGT activity in COS7 cells (Fig. 2). The cDNA for Bil UDPGT shares an identical 913-bp sequence (C-terminal 247 amino acid residues) with that for 4-NP UDPGT. Interestingly, this region includes the locus where a single base deletion occurs in the 4-NP UDPGT cDNA from the homozygous jaundiced Gunn rat (6). Both Bil and 4-NP UDPGTs are located on the same chromosome (7) and have genetic defects in the jaundiced Gunn rat (5,6). On the basis of these facts, we suppose that Bil and 4-NP UDPGT mRNAs are derived from a common primary-transcript. After an alternative splicing of the 5' end region of the transcript, the respective mature mRNAs may be produced. Thus, the multiple defects of UDPGT isoenzymes observed in the jaundiced Gunn rat may be caused by sharing a common 3' end

region on the transcript, which has a point mutation. We are now performing investigations that may prove our hypothesis.

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REFERENCES

1. Gunn, C. K. (1938) *J. Heredity*, **29**, 137-139
2. Lathe, G. H. and Walker, M. (1957) *Biochem. J.*, **67**, 9P
3. Schmid, R. and Hammaker, L. (1963) *J. Clin. Invest.*, **42**, 1720-1734
4. Arias, I. M., Gartner, L. M., Cohen, M., Ezzer, J. and Levi, A. J. (1969) *Am. J. Med.*, **18**, 395-409
5. Scragg, I., Celier, C. and Burchell B. (1985) *FEBS Lett.*, **183**, 37-42
6. Iyanagi, T., Watanabe, T. and Uchiyama, Y. (1989) *J. Biol. Chem.*, **264**, 21301-21307
7. Nagai, F., Homma, H., Tanase, H. and Matsui, M. (1988) *Biochem. J.*, **252**, 897-900
8. Iyanagi, T., Hanju, M., Sogawa, K., Fujii-Kuriyama, Y., Watanabe, S., Shively, J. E. and Anan, K. F. (1986) *J. Biol. Chem.*, **261**, 15607-15614
9. Maniatis, T., Fritsch, E. F. and Sambrook, J. (1982) *Molecular Cloning* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY)
10. Koiwai, O., Yokota, T., Kageyama, T., Hirose, T., Yoshida, S. Arai, K. (1986) *Nucl. Acid Res.*, **14**, 5777-5792
11. Jackson, M. R., Fournel-Gigleux, S., Harding, D. and Burchell, B. (1988) *Mol. Pharmacol.*, **34**, 638-642
12. Sanger, F., Nicklen, S. and Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 5463-5467