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Cloning of a human liver UDP-glucose pyrophosphorylase cDNA by complementation of the bacterial *galU* mutation

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A human liver cDNA clone which encodes the UDP-glucose pyrophosphorylase was isolated by complementation of a bacterial *galU* mutant. The deduced amino acid sequence of the human enzyme comprised 508 amino acids with a calculated molecular mass of 56,950. The human enzyme significantly resembles those of potato tuber and slime mold with a homology of 46.6% and 43.2%, respectively, in amino acid sequence. No homology was found between the eukaryotic and the prokaryotic enzymes. Northern blotting analysis revealed that the gene was expressed at the highest level in skeletal muscle, followed by liver, heart and kidney.

E. coli galU; Glycogen; UDP-glucose pyrophosphorylase cDNA

1. INTRODUCTION

Much of the carbohydrates that enter the hepatocyte are stored in the form of glycogen which functions as a reserve of glucose which can be rapidly utilized during fasting to maintain normoglycemia. The precursor for glycogen synthesis is a uridine diphosphate glucose (UDPG) converted from glucose-1-phosphate by a specific enzyme called UDPG pyrophosphorylase (UTP:glucose-1-phosphate uridylyltransferase) (EC 2.7.7.9). In a second reaction catalyzed by glycogen synthase (EC 2.4.1.21), the glucose from UDPG is attached to a glycogen primer through a (α1–4) bond, lengthening the glucose chain.

In addition to serving as a direct precursor for glycogen synthesis, UDPG is also required for normal galactose metabolism in organisms which utilize the Leloir pathway [1]. In Gram (−) bacteria, the UDPG pyrophosphorylase is encoded by the *galU* gene. The *Escherichia coli galU* mutants mimic the mutants defective in *gal* operon (*galETK*) in several ways such as increased sensitivity to galactose, inability to ferment galactose and synthesize polysaccharides [2,3]. Since the enzymatic properties of the human UDPG pyrophosphorylase is very similar to that of the bacterial enzyme [4–8], it is likely that the human cDNA clone encoding the UDPG pyrophosphorylase may be obtained by complementation of the *galU* defects in bacteria. We here report the successful isolation of a human UDPG pyrophosphorylase cDNA clone by such a strategy. The nucleotide sequence and expression pattern of this gene in various human organs were also determined.

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2. MATERIALS AND METHODS

2.1. *cDNA library screening*

A human liver cDNA library, constructed in the lambda ZAP vector unidirectionally in *Eco*RI–*Xho*I sites, was purchased from Stratagene (Cat. no. 937200, LaJolla, CA, USA). A phagemid library was generated from the library by in vivo excision as recommended by the supplier. *E. coli galU* mutant strain FF4001 (MC4100, *galU95*) was a generous gift of Dr. Svein Valla, University of Trondheim, Norway [9]. *E. coli* strain HU735 (C600, *F' lac::Tn5*), which was used as a donor of *F* factor, was obtained from Dr. Jonathan Ou in our department. MacConkey-galactose agar was prepared with MacConkey agar base (Difco Inc., Detroit, MI, USA) supplemented with 0.2% galactose. *E. coli* strain JM109 was routinely used as the recombinant DNA host.

2.2. *Recombinant DNA techniques and DNA sequencing*

All restriction enzymes and DNA modifying enzymes were obtained either from Promega (Madison, WI, USA) or Boehringer Mannheim (Germany) and used under the conditions recommended by the suppliers. All recombinant DNA experiments were carried out essentially as described [10]. The DNA fragments were subcloned into *M13mp18* and *mp19* and sequenced with Sequenase (US Biochemical, Cleveland, OH, USA) and [^{35}S]dATP (Amersham, Arlington Heights, IL, USA) as described by the manufacturer, with either the universal *M13* primer or synthetic oligonucleotides [11]. The sequence was confirmed in both strands, and the question of compressions was resolved by sequencing with dITP. Nucleotide sequence was analyzed with the DNASTAR program (DNASTAR Inc., Madison, WI, USA) using a Macintosh LC computer.

2.3. *Assay of UDPG pyrophosphorylase activity*

Bacteria were grown in Luria broth supplemented with 10 μM IPTG (isopropyl-β-D-thiogalactopyranoside) until OD_{600} = 1.0, then were harvested by centrifugation. The pellets were resuspended into a buffer containing 50 mM Tris-HCl, pH 7.8, 10 mM $MgCl_2$, and 1.0

mM 2-mercaptoethanol, and cells were disrupted by sonication. The lysate was clarified by centrifugation at 20,000 × *g* and protein concentration of the supernatant was determined by the method of Bradford [12] using the Bio-Rad Protein Assay. The assay procedure for UDPG pyrophosphorylase was modified from the procedure of Fujimura et al. [13] which was designed to determine the concentration of UDPG. The reaction mixture contains 50 mM Tris-HCl, pH 7.8, 10 mM $MgCl_2$, 1 mM 2-mercaptoethanol, 1.0 mM UTP, 1.0 mM glucose-1-phosphate, 1 mM NAD, 0.05 units UDPG dehydrogenase (Boehringer Mannheim, Germany) and cell extract, in a final volume of 0.5 ml. Reactions were performed at 37°C for 30 min and the reduction of NAD was measured spectrophotometrically at 340 nm. The activity was defined as the amount of UDPG synthesized per min per mg protein.

2.4. *Northern blotting analysis*

A Multiple Tissue Northern (MTN) blot was obtained from Clontech (Palo Alto, CA, USA). This blot was made with poly(A)$^+$ RNA from eight different tissues of human, electrophoresed on denaturing agarose gel and blotted onto charged modified nylon membrane. The hybridization procedure and preparation of ^{32}P-labeled DNA probes were performed essentially as described [10].

3. RESULTS AND DISCUSSION

3.1. *Cloning strategy*

Bacterial mutants defective in the UDPG pyrophosphorylase (GalU) are suitable for complementation cloning for two reasons. First, the *galU* defect prevents the mutants from growing on galactose-containing agar. This provides a convenient means to select the Gal$^+$ bacteria from the Gal$^-$ bacteria. Secondly, the ability of fermenting galactose, which requires a functional UDPG pyrophosphorylase, can be easily determined by the color of colonies using MacConkey-galactose agar. Upon transformation with a human liver cDNA library, any transformant of Gal$^+$ phenotype, as determined by its ability to grow into red colonies on McConkey-galactose plates, is presumed to contain a human UDPG pyrophosphorylase cDNA clone.

3.2. *Cloning of human cDNA capable of complementing the* E. coli galU *mutation*

A human liver cDNA library constructed in the lambda ZAP vector was used for screening for its capability of conversion into the phagemid form by in vivo excision. The phagemids can then be transfected into a *E. coli galU* mutant carrying the *F* plasmid with high efficiency. To construct a suitable phagemid recipient strain, *F* factor was mobilized from *E. coli* HU735 to *galU* mutant strain FF4001 via conjugation, resulting in *E. coli* HP069. About 5×10^4 recombinant phagemid clones were transfected *E. coli galU* mutant strain HP069. The transfected bacteria were washed once with 0.85% NaCl and plated onto MacConkey-galactose agar supplemented with 50 μg/ml of ampicillin. About 60 colonies were isolated, which were capable of fermenting galactose and manifested as red colonies on the MacConkey-galactose plates. To eliminate spontaneous revertants or contaminating bacteria, plasmid DNA was prepared from 12 of these Gal$^+$ clones and retransformed into *E. coli* HP069. All transformants of the 12 plasmids examined exhibited galactose insensitive phenotype and were positive in galactose fermentation. Transformants with the pBluescript vector alone remained Gal$^-$. These plasmids were further digested with restriction endonuclease *Eco*RI, and analyzed on a 2% agarose gel. All 12 plasmids yielded a DNA fragment with varying sizes ranging from 850 to 1,150 bp and a common 200 bp *Eco*RI restricted fragment. Hybridization of these plasmids with the 1,150 bp DNA fragment as a probe indicated all these samples share a common nucleotide sequence (data not shown). It is apparent that all these cDNA clones are derived from an identical gene, presumably the gene encoding the human liver UDPG pyrophosphorylase. The clone with the longest insert, designated pHC308, was selected and used for further study.

3.3. *Analysis of the biological activity of the pHC308 clone in* E. coli

In addition to the complementation study, more solid evidence supporting that pHC308 encodes a UDPG pyrophosphorylase activity came from the analysis of the enzymatic activity in the transformant. As demonstrated in Table I, the UDPG pyrophosphorylase activity in *E. coli* HP069[pHC308] is about 20 times higher than the activity in HP069[pBluescript]. Therefore, it can be concluded that pHC308 contains a human liver cDNA clone encoding a UDPG pyrophosphorylase.

3.4. *Nucleotide sequence analysis of human UDPG pyrophosphorylase cDNA clone*

The human UDPG pyrophosphorylase cDNA contained in the pHC308 comprised 1,984 nucleotides (Fig. 1). One major open reading frame was observed which is capable of coding a protein of 508 amino acid in length. The predicted molecular mass is 56,950 Da, which matches perfectly with the size of the purified bovine enzyme (Boehringer Mannheim) analyzed on SDS-polyacrylamide gel electrophoresis. The result is also in agreement with the previous report which showed that the molecular weight of the mammalian enzyme is approximately 400 kDa with eight identical subunits [14].

3.5. *Sequence comparison with UDPG pyrophosphorylase from other sources*

The UDPG pyrophosphorylase gene has been re-

Table I

UDPG pyrophosphorylase activity of the cDNA clone in *E. coli*

Escherichia coli strains	UDPG pyrophosphorylase activity (nmol/min/mg protein)
HP069[pBluescript]	12.5
HP069[pHC308]	251.4

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   1                                                    AATAGTTCCTTAAGTAGCTAACCATCGGAGGAGAAAGAACACATCGGTTGTTGCTTGA
  58  AAGGAAGGGAGACCTTCCTTATAACGCAACTCCGAGAAATTGAAAATTATAAACCAGATTATTTTAATATAACCCGAAA
   1    M    S    R    F    V    Q    D    L    S    K    A    M    S    Q    D    G    A    S    Q    F
 138  ATG  TCG  AGA  TTT  GTG  CAA  GAC  CTT  AGC  AAA  GCT  ATG  TCT  CAA  GAT  GGT  GCT  TCT  CAG  TTC
  21    Q    E    V    I    L    Q    E    L    E    L    S    V    K    K    E    L    E    K    I    L
 198  CAA  GAA  GTC  ATT  CTC  CAA  GAA  CTA  GAA  TTA  TCT  GTG  AAG  AAA  GAA  TTA  GAA  AAA  ATA  CTT
  41    T    T    A    T    S    H    E    Y    E    H    T    K    K    D    L    D    G    F    R    K
 258  ACA  ACA  GCA  ACC  TCA  CAT  GAG  TAT  GAG  CAT  ACT  AAA  AAA  GAT  CTT  GAT  GGA  TTT  CGG  AAG
  61    L    Y    H    R    F    L    Q    E    K    G    P    S    V    D    W    G    K    I    Q    R
 318  CTA  TAT  CAT  AGA  TTT  TTG  CAA  GAA  AAG  GGA  CCT  TCT  GTA  GAC  TGG  GGT  AAA  ATC  CAG  AGA
  81    P    P    E    D    S    I    Q    P    Y    E    K    I    K    A    R    G    L    P    D    N
 378  CCT  CCA  GAA  GAT  TCG  ATT  CAA  CCC  TAT  GAA  AAG  ATA  AAG  GCC  AGA  GGC  CTG  CCT  GAT  AAT
 101    I    S    S    V    L    N    K    L    V    V    V    K    L    N    G    G    L    G    T    S
 438  ATA  TCT  TCT  GTG  TTG  AAC  AAA  CTG  GTG  GTG  GTG  AAA  CTC  AAT  GGT  GGT  TTG  GGA  ACC  AGT
 121    M    G    C    K    G    P    K    S    L    I    G    V    R    N    E    N    T    F    L    D
 498  ATG  GGC  TGC  AAA  GGC  CCT  AAA  AGT  CTG  ATT  GGT  GTA  AGA  AAT  GAG  AAT  ACC  TTT  TTG  GAT
 141    L    T    V    Q    Q    I    E    H    L    N    K    S    Y    N    T    D    V    P    L    V
 558  CTA  ACT  GTG  CAG  CAA  ATT  GAA  CAT  TTG  AAC  AAA  AGC  TAC  AAT  ACA  GAT  GTC  CCT  CTT  GTT
 161    L    M    N    S    F    N    T    D    E    D    T    K    K    I    L    Q    K    Y    N    H
 618  TTA  ATG  AAC  TCT  TTT  AAC  ACG  GAT  GAA  GAC  ACA  AAA  AAA  ATA  CTT  CAG  AAG  TAC  AAT  CAT
 181    C    R    V    K    I    Y    T    F    N    Q    S    R    Y    P    R    I    N    K    E    S
 678  TGC  CGT  GTG  AAA  ATC  TAC  ACC  TTC  AAT  CAA  AGC  AGG  TAT  CCG  AGG  ATT  AAT  AAG  GAA  TCT
 201    L    R    P    V    A    K    D    V    S    S    S    G    E    S    T    E    A    W    Y    P
 738  TTA  CGG  CCT  GTA  GCA  AAG  GAT  GTG  TCT  TCC  TCA  GGG  GAA  AGT  ACA  GAA  GCT  TGG  TAC  CCC
 221    P    G    H    G    D    I    Y    A    S    F    Y    N    S    G    L    L    D    T    F    L
 798  CCA  GGA  CAT  GGA  GAT  ATC  TAT  GCT  AGT  TTC  TAC  AAC  TCG  GGC  TTG  CTC  GAC  ACC  TTT  CTA
 241    E    E    G    K    E    Y    I    F    V    S    N    I    D    N    L    G    A    T    V    D
 858  GAA  GAA  GGC  AAA  GAG  TAT  ATT  TTT  GTT  TCT  AAC  ATA  GAT  AAC  CTG  GGT  GCC  ACA  GTG  GAT
 261    L    Y    I    L    N    H    L    I    N    P    P    N    G    K    R    C    E    F    V    M
 918  CTT  TAT  ATT  CTT  AAT  CAT  CTA  ATC  AAC  CCA  CCC  AAT  GGG  AAA  CGC  TGT  GAA  TTT  GTC  ATG
 281    E    V    T    N    K    T    R    A    D    V    K    G    G    T    L    T    Q    Y    E    G
 978  GAA  GTC  ACA  AAT  AAA  ACA  CGA  GCA  GAT  GTA  AAG  GGT  GGA  ACA  CTC  ACT  CAA  TAT  GAA  GGC
 301    K    L    R    L    V    E    I    A    Q    V    P    K    A    H    V    D    E    F    K    S
1038  AAA  CTG  AGA  CTG  GTG  GAG  ATT  GCT  CAA  GTG  CCA  AAA  GCA  CAT  GTT  GAT  GAA  TTC  AAG  TCT
 321    V    S    K    F    K    I    F    N    T    N    N    L    W    I    S    L    A    A    V    K
1098  GTG  TCA  AAA  TTT  AAA  ATA  TTT  AAC  ACA  AAC  AAC  CTA  TGG  ATC  TCT  CTT  GCA  GCA  GTT  AAA
 341    R    L    Q    E    Q    N    A    I    D    M    E    I    I    V    N    P    K    T    L    D
1158  AGA  CTG  CAG  GAG  CAG  AAT  GCC  ATT  GAC  ATG  GAA  ATC  ATT  GTG  AAT  CCA  AAG  ACT  TTG  GAT
 361    G    G    L    N    V    I    Q    L    E    T    A    V    G    A    A    I    K    S    F    E
1218  GGA  GGC  CTG  AAT  GTT  ATT  CAG  TTA  GAA  ACT  GCA  GTT  GGA  GCT  GCA  ATT  AAA  AGT  TTC  GAG
 381    N    S    L    G    I    N    V    P    R    S    R    F    L    P    V    K    T    T    S    D
1278  AAT  TCT  TTA  GGT  ATT  AAT  GTT  CCA  AGG  AGT  CGT  TTT  CTG  CCT  GTG  AAG  ACC  ACG  TCA  GAT
 401    L    L    L    V    M    S    N    L    Y    S    L    N    A    G    S    L    T    M    S    E
1338  CTC  TTG  CTT  GTG  ATG  TCA  AAC  CTC  TAT  AGC  CTT  AAT  GCA  GGA  TCT  TTG  ACC  ATG  AGT  GAA
 421    K    R    E    F    P    T    V    P    L    V    K    L    G    S    S    F    T    K    V    Q
1398  AAG  CGG  GAG  TTT  CCT  ACA  GTA  CCC  TTG  GTT  AAA  TTA  GGA  AGT  TCC  TTT  ACC  AAG  GTT  CAA
 441    D    Y    L    R    R    F    E    S    I    P    D    M    L    E    L    D    H    L    T    V
1458  GAT  TAC  CTA  AGG  AGG  TTT  GAA  AGT  ATA  CCT  GAC  ATG  CTG  GAA  TTG  GAT  CAC  CTC  ACT  GTT
 461    S    G    D    V    T    F    G    K    N    V    S    L    K    G    T    V    I    I    I    A
1518  TCT  GGA  GAT  GTG  ACA  TTT  GGA  AAA  AAT  GTT  TCA  TTA  AAG  GGA  ACA  GTT  ATC  ATT  ATT  GCA
 481    N    H    G    D    R    I    D    I    P    P    G    A    V    L    E    N    K    I    V    S
1578  AAT  CAT  GGT  GAC  AGA  ATT  GAC  ATC  CCC  CCT  GGA  GCA  GTG  TTA  GAG  AAC  AAG  ATC  GTA  TCT
 501    G    N    L    R    I    L    D    H    •
1638  GGA  AAT  CTT  CGA  ATC  TTG  GAT  CAC  TAA  GATAAGCGCTGCCGGATACACTTTATACTAATTATGGGTTAAA
1708  CAGTTTCTTGTAATAAAATGTCCTCTAAGATTCTCAAATGAGCAAGTACTTTTACTGTGCAGTGTTGATTTTTTAGAGT
1795  TTTCTGCAATGTGCTTTTAGCCTAAAGAGAAGATAGATGGAGCAGTACTGTCCTTTTTTGATGAAGAGCCTAGAGATGA
1866  GTTCATCTAAAAGTGCAATATTATTTAATCGTAGAACTGGGCCAGCTCGGCAATCTTTTAACAGAAGCTGGAGTGATGG
1945  TCTTTGATTGCCTTTGATTTTAAAAATAAAGTGATACAAC
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Fig. 1. Nucleotide sequence and deduced amino acid sequence of human UDPG pyrophosphorylase. The nucleotide sequence is numbered in the 5′-to-3′ direction beginning at the first position of the *Eco*RI linker. The amino acid sequence is numbered from the first methionine at the putative translation initiation site. These data have been submitted to the GenBank data base and have been assigned accession number L14430.

ported from three evolutional-diversed organisms, *Acetobacter xylinum* [15], potato [16], and the slime mold, *Dictyostelium discoideum* [17]. The two eucaryotic proteins show significant (42%) sequence homology with each other. No homology was found between the bacterial enzyme and either of the two eucaryotic enzymes. By comparison of the human enzyme sequence with those of the same enzyme from these sources, significant homology of the human enzyme with the other two eucaryotic enzyme was noted (Fig. 2). A 43.2% identity (220 out of 508 matchable residues) was noted between the enzyme of human and of the slime mold and 46.6% (237 out of 508 residues) between the human enzyme and the enzyme from potato tuber. All these eucaryotic

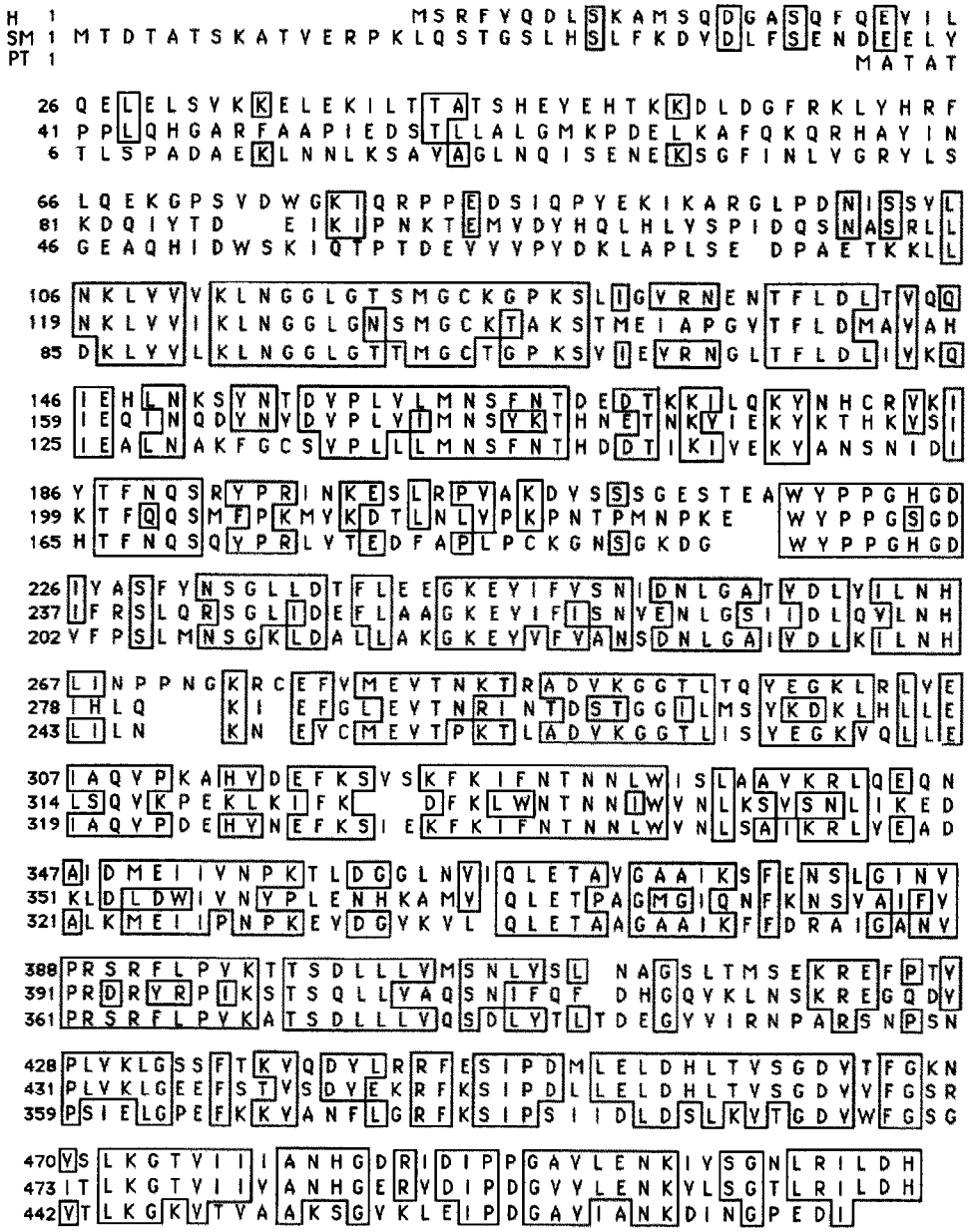

Fig. 2. Amino acid sequence comparison between human, potato tuber and slime mold UDPG pyrophosphorylases. The positions of the amino acid residues are indicated to the left. Residues identical with those of the human enzyme are boxed. The abbreviations are as follows: H, human; SM, slime mold; PT, potato tuber.

genes resemble one another over the entire sequence except about 100 amino acids at the N terminus where no homology was observed. In fact, much of these N-terminal sequences may not be critical for the catalytic activity of the human enzyme. One of the cDNA clones which encodes an enzyme truncated upto 43 amino acid residues at the N terminus remains able to complement the *galU* defect of *E. coli* mutant HP069. A site-specific mutagenesis has demonstrated that three lysyl residues are important for the normal function of the potato tuber UDPG pyrophosphorylase [18]. Two of the sites, Lys-367 and Lys-265 in the potato tuber enzyme, are also conserved in the human enzyme. Like the other two eucaryotic enzymes, no homology was observed between the human and bacterial UDPG pyrophosphorylase.

3.6. *Northern analysis of human UDPG pyrophosphorylase gene*

Except for the liver and ovary [19] in which relatively abundant UDPG pyrophosphorylase activity were noted, the distribution of the enzyme activity in different organs and tissue types is not clear. To investigate the expression level of this gene in various organs, the

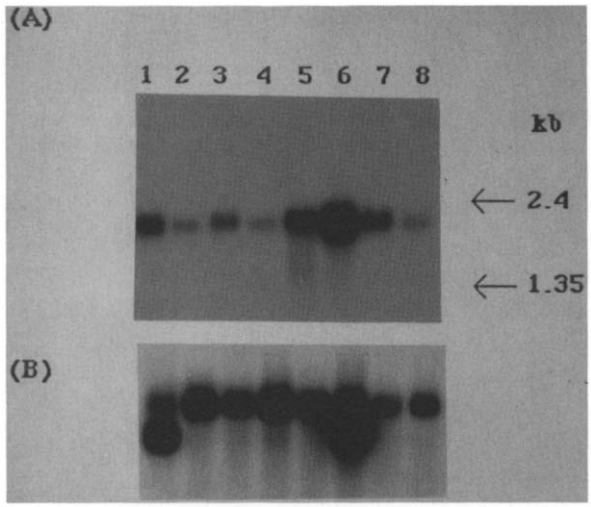

Fig. 3. Northern blotting analysis of the human UDPG pyrophosphorylase gene expression. Lanes 1, heart; 2, brain; 3, placenta; 4, lung; 5, liver; 6, skeletal muscle; 7, kidney; 8, pancreas. The probes used in this experiment are pHC308, the human UDPG pyrophosphorylase cDNA (A) and β-actin gene (B). The molecular weight markers are marked by arrows on the left.

cDNA insert was excised from pHC308, ^{32}P-labeled, and used as a probe to hybridize a Northern blot which contains poly(A) RNA from eight different human organ sources. As demonstrated in Fig. 3, all testing organs exhibit a single transcript of 2.2 kb in length comparable to the size of the cDNA clone. The result suggests that the pHC308 contains a nearly full length cDNA encoding the human UDPG pyrophosphorylase. This gene is found to express in all organs tested, with the highest level in skeletal muscle, followed by liver, heart, and kidney. By using a β-actin cDNA as a probe, the amount of poly(A) RNA of the skeletal muscle on the Northern blot was found to be slightly higher than in the other samples. Nevertheless, as measured by a densitometer, the amount of UDPG pyrophosphorylase gene transcript in skeletal muscle is still twice as much as that in liver. Our result is in agreement with the fact that in the human body, skeletal muscle and liver are the major organs that store glycogen. Further experiments are needed to confirm if the enzyme activities in these organs are indeed correlated with the expression level.

3.6. *Conclusion and prospects*

The UDPG pyrophosphorylase is an important metabolic enzyme which has been purified from plant [20,21] and animal [7,8] tissues, *E. coli* [4,5] and slime mold, *D. discoideum* [22]. In some species of Enterobacteriaceae, mutant strains with UDPG pyrophosphorylase deficiency are altered in the carbohydrate part of the lipopolysaccharides [2,3]. The alteration also affects the synthesis of bacterial flagella [23], the utilization of galactose [2], and osmoregulation [24]. The enzyme also participates in sucrose and cellulose formation in plants [25,26] and in bacteria [27] and the developmental regulation in *D. discoideum* [28]. In mammalian cells, the deficit of UDPG may result in decreased synthesis of membrane glycoproteins necessary for normal differentiation and maintenance of the integrity of cells. Furthermore, the defects of the UDPG pyrophosphorylase may cause abnormal galactose utilization and glycogen synthesis. Recent finding of the association between an *Xba*I restriction fragment length polymorphism of the glycogen synthase gene and non-insulin-dependent diabetes mellitus (NIDDM) [29] suggest that UDPG pyrophosphorylase, the rate-limiting enzyme of the glycogen biosynthesis pathway [30], may link to the disease as well. The human liver cDNA clone and its nucleotide sequence reported in this paper will provide useful information for further studies of the human UDPG pyrophosphorylase at the molecular biology level and its implications in NIDDM and related carbohydrate metabolic problems.

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