Cloning of a Novel Rat Gene, DB83, That Encodes a Putative Membrane Protein

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Abstract

Using a partial cDNA sequence and a 5'-RACE technique, we isolated a novel cDNA from rat liver referred to as DB83. DB83 had four hydrophobic trans-membrane domains and one N-myristoylation site as well as multiple possible phosphorylation sites. The db83 gene was highly expressed in the liver and significantly in brain, lungs and kidneys. We suggest that DB83 is a tissue-specific putative membrane protein.

Key words: cDNA cloning; membrane protein; tissue-specific gene expression

We have studied genes which are expressed abundantly in the liver. In a previous work, we obtained 13 partial cDNA inserts from rat liver by cDNA subtraction between normal liver and hepatoma using oligo(dT)-immobilized Latex beads.¹ One of those inserted, named DB83, was about 500 bp in length. Afterward, this region was mapped in the DB83 3'-untranslated region from 1308 to 1795 (Fig. 1). Northern blot analysis using this short probe provided multiple signals around 2.0 kb (data not shown). In our cDNA cloning protocol using subtraction,¹ cDNA inserts were cloned in pBluescript with EcoRI and XhoI. Since the original DB83 insert was found to be partial, we carried out 5'-RACE using two primers [nucleotides 1438 to 1458 and 1343 to 1365 (Fig. 1)] and a normal rat liver-derived 5'-RACE cDNA library (Clontech) to isolate a full-length cDNA. We obtained several RACE-derived cDNAs carrying DB83 sequences, and eventually connected them. The resultant long cDNA, that was found to carry an internal EcoRI site, and normal rat liver-derived 5'-RACE cDNA library (Clontech) to isolate a full-length cDNA. We obtained several RACE-derived cDNAs carrying DB83 sequences, and eventually connected them. The resultant long cDNA, that was found to carry an internal EcoRI site, contained one open reading frame (ORF) with 247 amino acids (Fig. 1). Homology searches with the connected cDNA and its corresponding ORF by the BLAST program revealed no homologous gene. We thus conclude that DB83 is a novel gene.

Several motif search programs (SOSUI, PROST and MOTIF) for DB83 amino acid sequence assigned four tracts with hydrophobic amino acids characteristic of trans-membrane domain (Fig. 1). We further found one N-myristoylation site at which a 14C-saturated fatty acid could be covalently attached.² More detailed homology search demonstrated that DB83 had weak and partial similarities with genome project-based hypothetical proteins of Synechocystis species,³ Saccharomyces cerevisiae,⁴ and Caenorhabditis elegans,⁴ and some of these proteins also had putative transmembrane domains. These results suggested that DB83 may be a novel type of membrane protein. Moreover, DB83 had putative multiple phosphorylation sites for protein kinases [⁵ and ⁶] (Fig. 1).

We examined tissue distribution of DB83 mRNA by Northern blot analysis (Fig. 2). The amounts of each RNA on the membrane was confirmed to be similar by the β-actin probe (Fig. 2, lower panel). In the liver, the 2.0-kb transcript was predominantly expressed (Fig. 2, lane 5). As seen in Fig. 2, this transcript was strongly expressed in the liver (lane 5), but weakly in the brain, lungs, and kidneys (lanes 2, 4 and 7). The 2.0-kb transcript was almost undetectable in the heart, spleen, skeletal muscle, and testes (Fig. 2, lanes 1, 3, 6, and 8, respectively). Moreover, we observed significant amounts of larger (5.5 and 6.5-kb) transcripts in the brain, spleen, lungs, liver, and kidneys. No significant signals were observed in the heart, skeletal muscle or testes (Fig. 2, lanes 1, 6, and 8). Because the connected DB83 cDNA
was 1.8-kb in size and contained a poly(dA) stretch (Fig. 1), we presume that the 2.0-kb signal represented bona fide DB83 mRNA. However, since the 1.8-kb cDNA was obtained by PCR, a possibility of the existence of longer DB83-related mRNAs could not be eliminated. The larger transcripts may be attributable to alternative splicing, the existence of family proteins, or different transcription start sites. Thus, DB83 may play an important role in the liver, in addition to other DB83-expressing tissues. This gene was obtained by cDNA subtraction between normal liver and hepatoma, and the possibility of a relationship between DB83 and hepatocarcinogenesis remains to be investigated.

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References
