Cloning and expression of a human choline/ethanolaminephosphotransferase: synthesis of phosphatidylcholine and phosphatidylethanolamine

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Cholinephosphotransferase catalyses the final step in the synthesis of phosphatidylcholine (PtdCho) via the Kennedy pathway by the transfer of phosphocholine from CDP-choline to diacylglycerol. Ethanolaminephosphotransferase catalyses an analogous reaction with CDP-ethanolamine as the phosphobase donor for the synthesis of phosphatidylethanolamine (PtdEtn). Together these two enzyme activities determine both the site of synthesis and the fatty acyl composition of PtdCho and PtdEtn synthesized *de novo*. A human choline/ethanolaminephosphotransferase cDNA (hCEPT1) was cloned, expressed and characterized. Northern blot analysis revealed one hCEPT1 2.3 kb transcript that was ubiquitous and not enriched, with respect to actin, in any particular cell type. The open reading frame predicts a protein (hCEPT1p) of 416 amino acid residues with a molecular mass of 46550 Da containing seven membrane-spanning domains. A predicted amphipathic helix resides within the active site of the enzyme with the final two aspartic residues of the CDP-alcohol phosphotransferase motif, $DG(X)_2AR(X)_8G(X)_3D(X)_3D$, positioned within this helix. hCEPT1p was successfully expressed in a full-length, active form in *Saccharomyces cerevisiae* cells devoid of endogenous cholinephosphotransferase or ethanolaminephosphotransferase activities (HJ091, *cpt1::LEU2 ept1*$^-$). *In vitro*, hCEPT1p displayed broad substrate specificity, utilizing both CDP-choline and CDP-ethanolamine as phosphobase donors to a broad range of diacylglycerols, resulting in the synthesis of both PtdCho and PtdEtn. *In vivo*, *S. cerevisiae* cells (HJ091, *cpt1::LEU2 ept1*$^-$) expressing hCEPT1 efficiently incorporated both radiolabelled choline and ethanolamine into phospholipids, demonstrating that hCEPT1p has the ability to synthesize both choline- and ethanolamine- containing phospholipids *in vitro* and *in vivo*.

Key words: cholinephosphotransferase, ethanolaminephosphotransferase, lipid, membrane, phospholipid.

INTRODUCTION

Cholinephosphotransferase catalyses the final step in the synthesis of phosphatidylcholine (PtdCho) through the Kennedy pathway via the transfer of a phosphocholine moiety from CDP-choline to diacylglycerol, with the release of CMP and the formation of PtdCho [1–7]. Ethanolaminephosphotransferase catalyses an analogous reaction with CDP-ethanolamine as the phosphobase donor for the synthesis of phosphatidylethanolamine (PtdEtn). The fatty acyl composition of the diacylglycerol molecule utilized by these enzyme activities determines the fatty acyl array for PtdCho and PtdEtn synthesized *de novo*. In addition, their intracellular location identifies the site of PtdCho and PtdEtn synthesis *de novo* for subsequent transfer to other organelles, or for assembly with proteins and other lipids for secretion during the synthesis of lung surfactant, lipoproteins and bile [8–13].

Genetic approaches led to the isolation of two genes encoding cholinephosphotransferase or ethanolaminephosphotransferase activities from the yeast *Saccharomyces cerevisiae*. The yeast *CPT1* gene product encodes a cholinephosphotransferase (Cpt1p) [6,14,15] specific for the synthesis of PtdCho *in vitro* and *in vivo*, whereas the *S. cerevisiae* gene *EPT1* product codes for a dual-specificity choline/ethanolaminephosphotransferase (Ept1p) capable of synthesizing PtdCho and PtdEtn *in vitro* but that synthesizes primarily PtdEtn *in vivo* [6,16]. Analysis of chimaeric *CPT1*/*EPT1* enzymes [6,16,17] mapped the active-site domain, and site-directed mutagenesis identified a diagnostic catalytic motif [18].

The precise roles of the various proposed mammalian isoforms of cholinephosphotransferase or ethanolaminephosphotransferase in the regulation of the partitioning of lipid biosynthetic pathways have yet to be determined because a mammalian cholinephosphotransferase or ethanolaminephosphotransferase has yet to be cloned or purified, although a guinea-pig short cDNA library PCR product (0.1 kb) with similarity to the yeast *CPT1* gene has been reported [19]. Here we describe the first isolation of a cDNA coding for a mammalian choline/ethanolaminephosphotransferase (hCEPT1), its heterologous expression and its characterization *in vitro* and *in vivo*.

EXPERIMENTAL

Materials

[α-^{32}P]dCTP (3000 Ci/mmol) and [*methyl*-^{14}C]choline chloride were purchased from DuPont/NEN. [*methyl*-^{14}C]CDP-choline was purchased from both American Radiolabelled Chemicals and Amersham Pharmacia. [1,2-^{14}C]Ethanolamine hydrochloride and [*ethanolamine*-1,2-^{14}C]CDP-ethanolamine were products of ICN. *Taq* polymerase and custom oligonucleotides were purchased from Life Technologies. Lipids were products of Avanti Polar Lipids except for $C_{16:0}/C_{22:6}$ PtdCho and $C_{18:0}/C_{22:6}$ PtdCho, which were purchased from Sigma. All other reagents were of the highest quality available commercially.

Abbreviations used: AAPT1p, plant CDP-aminoalcoholphosphotransferase protein; *CPT1*, *Saccharomyces cerevisiae* cholinephosphotransferase gene; Cpt1p, *S. cerevisiae* cholinephosphotransferase protein; *EPT1*, *S. cerevisiae* choline/ethanolaminephosphotransferase gene; Ept1p, *S. cerevisiae* choline/ethanolaminephosphotransferase protein; EST, expressed sequence tag; hCEPT1, human choline/ethanolaminephosphotransferase cDNA; hCEPT1p, human choline/ethanolaminephosphotransferase protein; PtdCho, phosphatidylcholine; PtdEtn, phosphatidylethanolamine.

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Isolation and expression of the full-length hCEPT1

A TBLASTN search [20] was performed against the Expressed Sequence Tag (EST) database with the use of default parameters with reference to the predicted amino acid sequences of both the entire *S. cerevisiae* Cpt1p-coding region and the Cpt1p CDP-alcohol phosphotransferase motif [18]. Several ESTs were identified that possessed similarity to either the Cpt1p CDP-alcohol phosphotransferase motif or to other regions within the Cpt1p protein. These ESTs were obtained and sequenced. One cDNA (human Jurkat T cell, Genbank accession number AA312638) contained a full-length open reading frame with a high degree of similarity to the yeast Cpt1p, including a diagnostic CDP-alcohol phosphotransferase motif region [18]. This cDNA was sequenced in its entirety on both strands (Genbank accession no. AF068302). A proposed coding region within this EST was amplified by PCR and subcloned into the *Bam*HI/*Sal*I sites of the *Escherichia coli* expression vector pET23a (Novagen), resulting in the addition of an 11-residue T7 epitope tag [21] to the N-terminus of the protein (pAH5). The T7-tagged version of hCEPT1 was excised from pAH5 with *Bgl*II and *Sal*I and subcloned into the constitutive *S. cerevisiae* expression vector p416 GPD [22], creating pAH9. All PCR-derived products were also sequenced in their entirety.

Northern blot

Random-primed ^{32}P-labelled probes [23] were synthesized by using either the entire 1.2 kb coding region of hCEPT1 or a 2.0 kb region of human β-actin cDNA. Multiple human tissue Northern blots (Clontech) were hybridized at 68 °C in Express-Hyb solution [24] for 1 h and washed in accordance with the manufacturer's instructions. Blots were exposed to X-ray film for 1–3 days.

Western blot

Membranes were prepared from BL21(DE3)*pLysS E. coli* and HJ091 *S. cerevisiae* cells (*cpt1::LEU2 ept1*$^-$) [18] grown to mid-exponential phase in appropriate medium to ensure plasmid maintenance [25]. The HJ091 strain of *S. cerevisiae* is devoid of endogenous cholinephosphotransferase or ethanolaminephosphotransferase activity owing to inactivated alleles at the loci encoding for these activities (*cpt1::LEU2 ept1*$^-$). Proteins were transferred to PVDF membranes [18] and blots were probed with a T7 epitope tag-specific monoclonal antibody (1:5000 dilution; Novagen) coupled to horseradish peroxidase for subsequent detection with the enhanced chemiluminescence (ECL®; Amersham) system.

Enzyme assays

Cholinephosphotransferase and ethanolaminephosphotransferase activities were determined from membrane preparations of HJ091 *S. cerevisiae* cells (*cpt1::LEU2 ept1*$^-$) [18] transformed with p416 GPD (mock) or pAH9 (hCEPT1) by the method of O et al. [26]. Diacylglycerols were dried under nitrogen gas and resuspended in 0.015% (w/v) Tween 20 by sonication. Unless indicated otherwise, assay buffer contained 100 mM Tris/HCl, pH 8.0, 20 mM $MgCl_2$, 1 mM EDTA, 1 mM diacylglycerols [final Tween 20 concentration 0.00375% (w/v)] and 10 μg of microsomal protein. Components were incubated at room temperature for 5 min; CDP-choline or CDP-ethanolamine (0.2 mM, 2000 dpm/nmol) was then added. Assays were incubated at 37 °C for 15 min and were terminated by the addition of 3 ml of chloroform/ethanol (2:1, v/v) followed by 1.5 ml of 0.9% KCl. Tubes were vortex-mixed and phase separation was facilitated by centrifugation at 2000 ***g*** for 10 min. The aqueous phase was aspirated and the organic phase was washed twice with 1.5 ml of 40% (v/v) ethanol. An aliquot of the organic phase was dried in a scintillation vial and radioactivity was determined. Samples were routinely analysed by TLC on silica gel plates in a solvent system of chloroform/methanol/NH_4OH/water (70:30:4:2, by vol.) to confirm products. In the absence of exogenous diacylglycerol, pAH9-directed cholinephosphotransferase activity was 0.55 nmol/min per mg and ethanolaminephosphotransferase activity was 0.81 nmol/min per mg; these values are due to endogenous diacylglycerols present in the membrane preparation and were subtracted from all measured activities.

Metabolic labelling

S. cerevisiae HJ091 cells (*cpt1::LEU2 ept1*$^-$) transformed with p416 GPD (mock) or pAH9 (hCEPT1) were grown to mid-exponential phase in synthetic dextrose medium containing appropriate nutritional supplements to ensure plasmid maintenance [25]. [^{14}C]Choline (10 μM, 10^5 d.p.m./nmol) was added to the cultures for 1 h. For [^{14}C]ethanolamine experiments, the cells were washed twice in synthetic dextrose medium plus required nutritional supplements but without $(NH_4)_2SO_4$, and resuspended in media lacking $(NH_4)_2SO_4$ before the addition of [^{14}C]ethanolamine (6.7 μM, 2.2×10^5 d.p.m./nmol) for 1 h. The reduced-nitrogen medium was required for the efficient uptake of ethanolamine. After incubation with radiolabel, cells were concentrated by centrifugation, washed twice with water and resuspended in 1 ml chloroform/methanol (1:1, v/v). Cells were disrupted for 1 min at 4 °C with a BioSpec Multi Bead Beater containing 0.5 g of 0.5 mm acid-washed glass beads. The beads were washed with 1.5 ml of chloroform/methanol (2:1, v/v). To facilitate phase separation, water and chloroform were added. Phospholipids in the organic phase were analysed by TLC on Whatman silica gel 60A plates with the solvent system chloroform/methanol/water/acetic acid (70:30:4:2, v/v). Aqueous metabolites were concentrated under vacuum, resuspended in water and separated by TLC on Whatman silica gel 60A plates. Choline-containing metabolites were separated in a solvent system consisting of ethanol/0.6% NaCl/NH_4OH (50:50:5, v/v). Ethanolamine-containing metabolites were separated with ethanol/2% (v/v) NH_4OH (1:2, v/v). Radiolabel was detected with a BIOSCAN System 200 imaging scanner to identify and integrate the radioactive bands.

Protein and lipid determination

Protein was determined by the method of Lowry et al. [27]. Diacylglycerols not available commercially were prepared from the representative PtdCho by phospholipase C digestion; their masses were determined by the method of Priess et al. [28].

RESULTS

Analysis of the hCEPT1 Product

The hCEPT1 coding region predicts a protein (hCEPT1p) of molecular mass 46550 Da composed of 416 amino acid residues, of which 48.6% are hydrophobic. No signal or sorting sequences were apparent. An expression system devoid of endogenous cholinephosphotransferase and ethanolaminephosphotransferase activities was sought for subsequent enzymological analysis of hCEPT1p. Two systems were explored: (1) prokaryotes are devoid of the Kennedy pathways, so hCEPT1 was expressed in *E. coli*; (2) hCEPT1 was produced in *S. cerevisiae* strain HJ091 (*cpt1::LEU2 ept1*$^-$), which contains null mutations at the loci coding for its endogenous cholinephosphotransferase (*CPT1*)

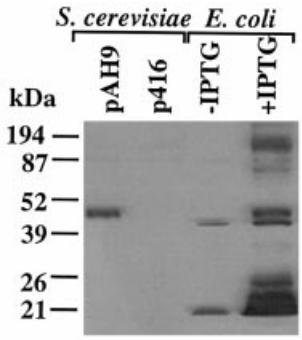

Figure 1 Western blot of hCEPT1p expressed in *S. cerevisiae* and *E. coli*

Membrane preparations from *S. cerevisiae* strain HJ091 (*cpt1::LEU2 ept1*$^-$) containing pAH9 or p416 GPD, or from *E. coli* BL21(DE3) *pLysS* containing pAH5 (with or without induction of protein with 0.4 mM isopropyl β-D-thiogalactoside for 2 h at 25 °C) were separated by SDS/PAGE and blotted with a monoclonal antibody against the T7 epitope as described in the Experimental section. The positions of molecular mass markers are indicated at the left.

Table 1 Cholinephosphotransferase and ethanolaminephosphotransferase activities of hCEPT1p

Enzyme activities were determined in microsomal membranes from *S. cerevisiae* strain HJ091 (*cpt::LEU2 ept1*$^-$) expressing hCEPT1 from pAH9 as described in the Experimental section. Results are means ± S.E.M. for three separate experiments performed in duplicate. Abbreviation: n.d., not detectable.

		Enzyme activity (nmol/min per mg of protein)	
Diacylglycerol	Substrate ...	CDP-choline	CDP-ethanolamine
Di-$C_{8:0}$		11.0 ± 1.3	n.d.
Di-$C_{10:0}$		345 ± 2.0	n.d.
Di-$C_{12:0}$		5.5 ± 1.4	n.d.
Di-$C_{14:0}$		1.4 ± 0.05	n.d.
Di-$C_{16:0}$		0.9 ± 0.03	n.d.
Di-$C_{16:1}$		14.2 ± 1.7	1.8 ± 0.2
Di-$C_{18:1}$		10.3 ± 0.5	4.0 ± 0.2
$C_{16:0}/C_{18:1}$		7.5 ± 0.4	2.0 ± 0.2
$C_{16:0}/C_{22:6}$		10.3 ± 0.7	1.0 ± 0.1
$C_{18:0}/C_{22:6}$		2.5 ± 0.1	n.d.

and choline/ethanolaminephosphotransferase (*EPT1*) [16,18]. Western blot analysis of T7 epitope-tagged hCEPT1p revealed that one band of the expected molecular mass, 46 550 Da, was produced in both organisms (Figure 1). All of the hCEPT1p detected in *S. cerevisiae* was full-length; however, most of the hCEPT1p produced in *E. coli* had undergone proteolytic degradation. Therefore heterologous expression of hCEPT1 in *S. cerevisiae* HJ091 was utilized for subsequent analyses.

Previous studies, using both crude and partly purified membrane preparations *in vitro* as well as metabolic labelling experiments *in vivo*, have described that cholinephosphotransferase activity prefers unsaturated and mono-unsaturated diacylglycerols as substrates, whereas ethanolaminephosphotransferase prefers diacylglycerols with polyunsaturated fatty acids at the *sn*-2 position with particular preference for hexaenoic ($C_{22:6}$) species [29–32]. Obvious differences in the efficacy of hCEPT1p in catalysing phosphobase transfer from either CDP-choline or CDP-ethanolamine to various diacylglycerols were apparent (Table 1). Of the diacylglycerols tested, CDP-choline preferred di-$C_{10:0} \gg$ di-$C_{16:1}$ > di-$C_{8:0}$ = di-$C_{18:1}$ = $C_{16:0}/C_{22:6}$, whereas

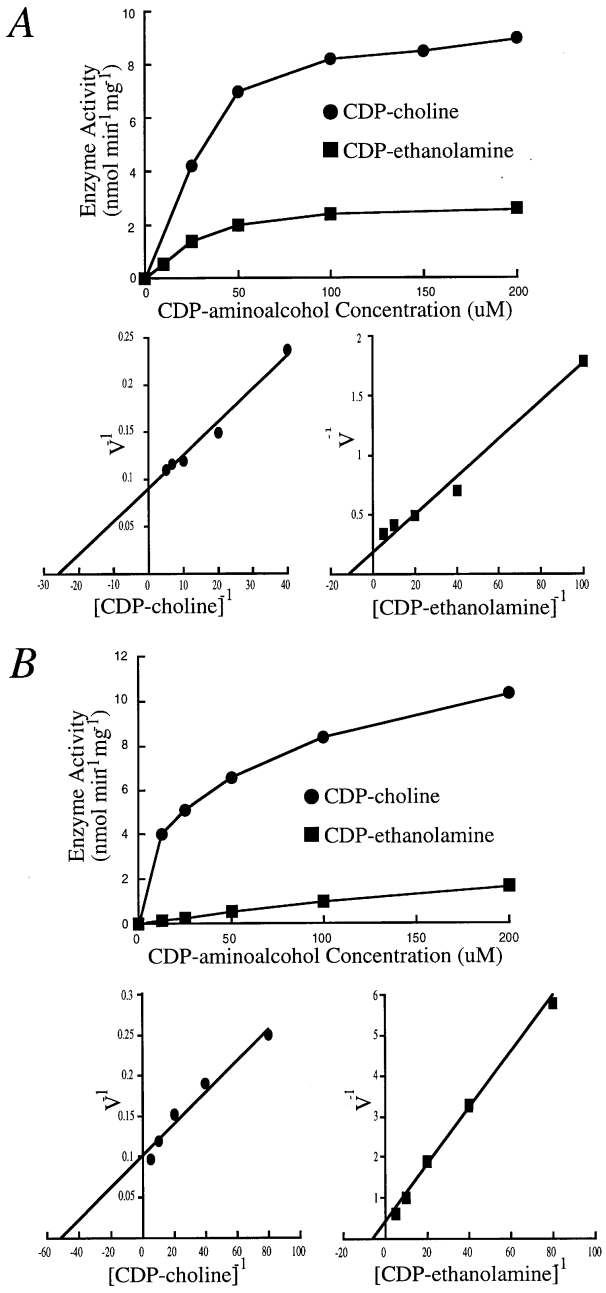

Figure 2 Kinetic analysis of hCEPT1p

Enzyme activities were determined in microsomal membranes from *S. cerevisiae* cells (HJ091, *cpt1::LEU2, ept1*$^-$) expressing hCEPT1p from pAH9 essentially as described in the Experimental section except for the CDP-aminoalcohol concentrations, which were as illustrated. Results are the means for four experiments. Di-$C_{18:1}$ diacylglycerol (**A**) and $C_{16:0}/C_{22:6}$ diacylglycerol (**B**) were used as substrates.

CDP-ethanolamine preferred di-$C_{18:1}$ > di-$C_{16:1}$ = $C_{16:0}/C_{18:1}$ = $C_{16:0}/C_{22:6}$. A kinetic analysis of hCEPT1p with di-$C_{18:1}$ as the diacylglycerol substrate in the presence of either CDP-choline or CDP-ethanolamine revealed a K_m (app) of 37 μM and a V_{max} (app) of 10.5 nmol/min per mg for CDP-choline, and a K_m (app) of 101 μM and a V_{max} (app) of 4.35 nmol/min per mg for CDP-ethanolamine (Figure 2). Similar results were obtained with $C_{16:0}/C_{22:6}$ diacylglycerol as a substrate, with a K_m (app) of

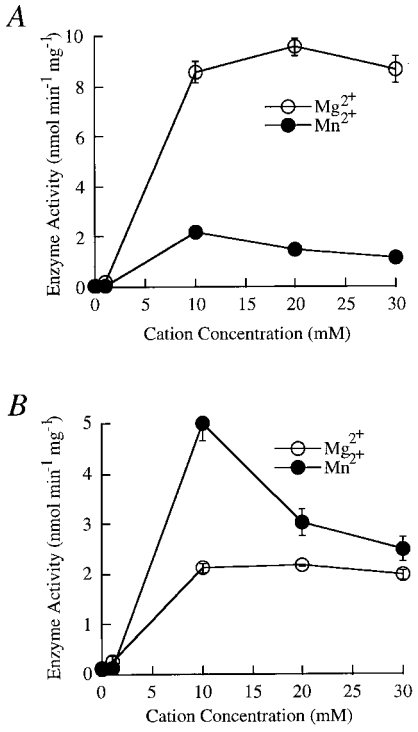

Figure 3 Cation requirements of hCEPT1p

Enzyme activities were determined in microsomal membranes from *S. cerevisiae* cells (HJ091, *cpt1::LEU2, ept1*$^-$) expressing hCEPT1p from pAH9 essentially as described in the Experimental section except for cation concentrations, which were as illustrated. Results are means ± S.E.M. for four experiments.

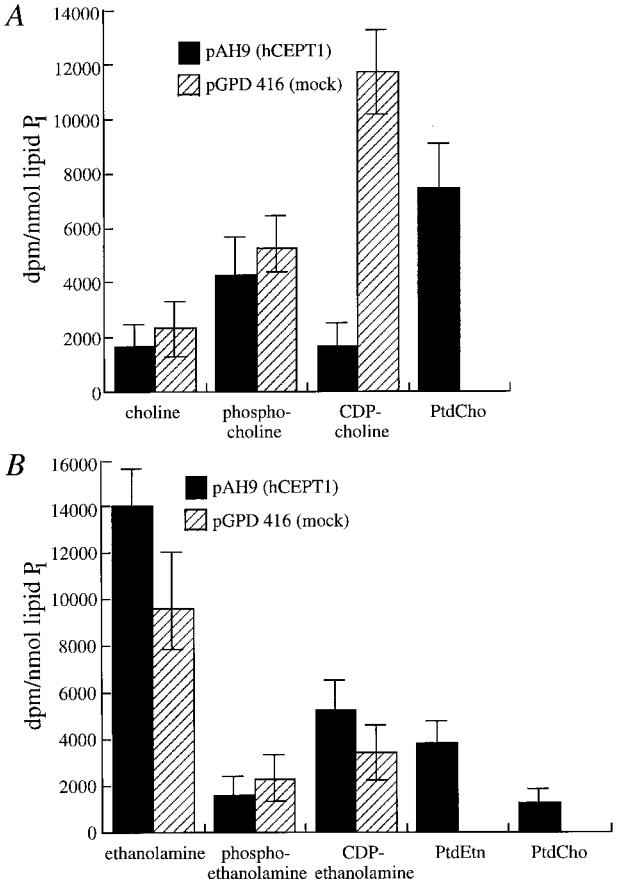

Figure 4 Metabolic labelling of hCEPT1 expressed in *S. cerevisiae* devoid of cholinephosphotransferase and ethanolaminephosphotransferase activities

Radiolabelled choline (**A**) or ethanolamine (**B**) was added to exponential-phase *S. cerevisiae* (HJ091, *cpt1::LEU2 ept1*$^-$) cells for 1 h. The incorporation of radiolabel into phospholipids, and also the metabolites of the CDP-choline and CDP-ethanolamine pathways, was monitored and quantified as described in the Experimental section. Results are means ± S.D. for at least four separate experiments.

28 μM and a V_{max} (app) of 10.1 nmol/min per mg for CDP-choline, and a K_m (app) of 133 μM and a V_{max} (app) of 2.41 nmol/min per mg for CDP-ethanolamine.

Cation requirements

Several investigations attempting to purify mammalian cholinephosphotransferases or ethanolaminephosphotransferases have resulted in the resolution of separate peaks of activities with various CDP-aminoalcohol specificities that differed in their abilities to use Mg^{2+} or Mn^{2+} as the essential cation [4,26,33]. The ability of hCEPT1p to utilize both CDP-choline and CDP-ethanolamine as substrates prompted the examination of its ability to use Mg^{2+} or Mn^{2+} as the essential cation cofactor with respect to each substrate (Figure 3). Both activities of hCEPT1 required millimolar levels of each cation for activity to be detected. Mg^{2+} activated the cholinephosphotransferase activity of hCEPT1p to a 4-fold greater extent than Mn^{2+}, whereas the ethanolaminephosphotransferase activity was preferentially activated by Mn^{2+} at 10 mM (2.5-fold more activity than Mg^{2+}) but was inhibited at higher Mn^{2+} concentrations, resulting in activities that approached those of Mg^{2+} activation. These are essentially identical results with those observed by Kanoh and Ohno [33] for a partially purified choline/ethanolaminephosphotransferase activity from rat liver. The precise mechanism by which cations activate cholinephosphotransferases or ethanolaminephosphotransferases has yet to be resolved; both cation–CDP-aminoalcohol and cation–enzyme complexes have been proposed. Work on chimaeric yeast Cpt1p/Ept1p enzymes predicted that Mg^{2+} was used *in vivo* [16,17]; however, resolution awaits the availability of pure protein.

Metabolic labelling

To test whether the *in vitro* cholinephosphotransferase and ethanolaminephosphotransferase activities catalysed by hCEPT1p could be recapitulated *in vivo*, radiolabelled choline or ethanolamine was added to exponential-phase *S. cerevisiae* cells devoid of endogenous cholinephosphotransferase or ethanolaminephosphotransferase activity (HJ091, *cpt1::LEU2 ept1*$^-$) transformed with either p416 GPD (mock) or pAH9 (hCEPT1 in p416 GPD). As seen in Figure 4, the addition of radiolabelled choline to cells expressing hCEPT1 resulted in the reconstitution of the CDP-choline pathway for the synthesis of PtdCho. In addition, the expression of hCEPT1 resulted in the synthesis of PtdEtn from radiolabelled ethanolamine, with subsequent conversion of some of the radiolabelled PtdEtn to PtdCho via the active PtdEtn methylation pathway present in *S. cerevisiae*. These results demonstrate that the dual specificity of hCEPT1p for CDP-choline and CDP-ethanolamine observed *in vitro* is also

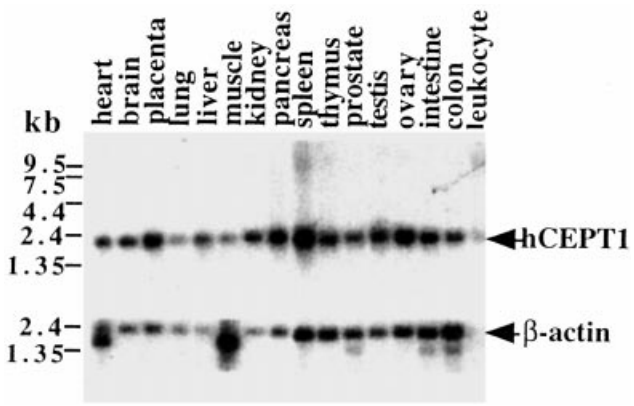

Figure 5 Northern blot analysis of hCEPT1 and β-actin transcripts in human cell types

Northern blots were probed at high stringency as described in the Experimental section. The positions of molecular mass markers are indicated at the left.

reflected *in vivo*. In addition, these results demonstrate that the *S. cerevisiae* and human forms of cholinephosphotransferases and ethanolaminephosphotransferases are of significant functional similarity to allow the reconstitution of both the CDP-choline and CDP-ethanolamine pathways in yeast by the human enzyme.

Transcript levels

A choline/ethanolaminephosphotransferase capable of synthesizing PtdCho and PtdEtn would be predicted to be expressed in all tissues. To assess the tissue distribution of hCEPT1, a multiple human tissue Northern blot was hybridized at high stringency with (1) a random primed probe synthesized by using the entire 1.2 kb hCEPT1 coding region, and (2) a 2.0 kb region of human β-actin. One hCEPT1 transcript of 2.3 kb was observed in all tissue examined; there was no obvious enrichment in any one cell type when normalized to β-actin content (Figure 5).

Structural predictions

The CDP-aminoalcohol phosphotransferases Cpt1p and Ept1p from yeast [14,15], and plant CDP-aminoalcoholphosphotransferase protein (AAPT1p) from soybean [34], were aligned with hCEPT1p (Figure 6). Overall identities and similarities as compared with hCEPT1p were: 23.1% and 38.2% for yeast Cpt1p; 21.9% and 34.6% for yeast Ept1p and 26.9% and 41.8% for AAPT1p. Inspection revealed that the CDP-alcohol phosphotransferase motif, $DG(X)_2AR(X)_8G(X)_3D(X)_3D$, spans

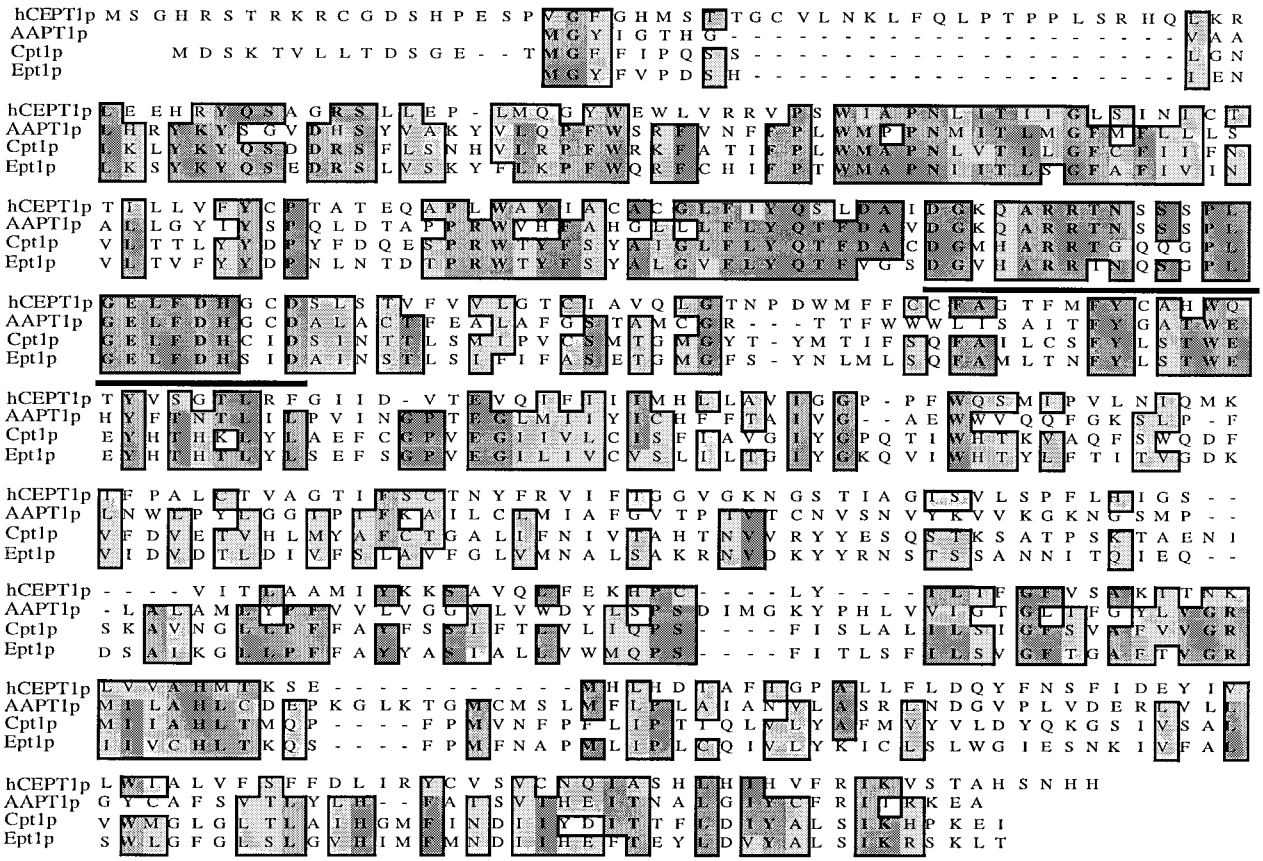

Figure 6 Alignment of hCEPT1p with known choline/ethanolaminephosphotransferases

The CLUSTAL-W alignment algorithm, set at default parameters, was used. Underlined residues indicate the positioning of the catalytic CDP-alcohol phosphotransferase motif.

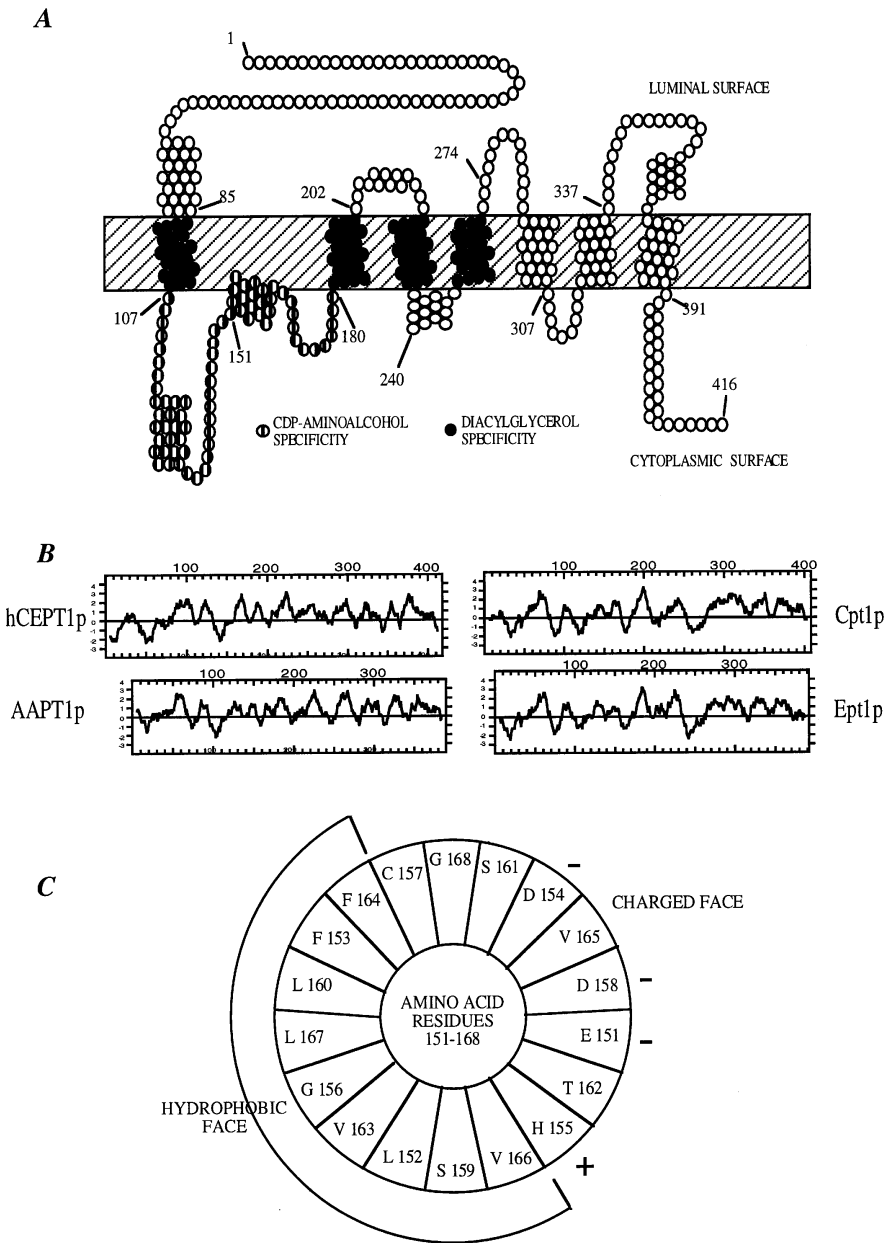

Figure 7 Predicted secondary structure of hCEPT1p

(**A**) The secondary structure of hCEPT1p was predicted by using the TMpred algorithm [35] to determine membrane-spanning domains. Two separate secondary structure algorithms, nnpredict and PHD [42,43], were used to estimate the location of α-helix and β-sheet secondary structures in hCEPT1p. (**B**) Kyte Doolittle plots for each of the known CDP-aminoalcoholphosphotransferase enzymes. (**C**) The positioning of amino acid residues of hCEPT1p in a predicted amphipathic helix within the catalytic domain.

residues 136–158 of hCEPT1p and is located in a similar position within the primary sequence of each enzyme (Figure 6).

Membrane-spanning domains for each enzyme were estimated by using the TMpred algorithm [35] and positioned within each sequence. A strong membrane-spanning helix prediction within three out of four of the aligned CDP-aminoalcohol phosphotransferases was the criterion used to position each bilayer-spanning region (Figure 7A). Kyte Doolittle plots are very similar for hCEPT1p, AAPT1p, Cpt1p and Ept1p (Figure 7B), consistent with the high degree of similarity between each of these enzymes throughout their primary sequences and with similar predicted higher-level structures for each enzyme. Of notable interest is a predicted amphipathic helix within a similar region of the active site of each enzyme (Figure 7C); the final two aspartic residues of the CDP-alcohol phosphotransferase motif, the known catalytic residues within the *S. cerevisiae* Cpt1p enzyme, reside within this amphipathic helix.

DISCUSSION

This study describes the first isolation and expression of a mammalian cDNA, hCEPT1, coding for either a choline-phosphotransferase or an ethanolaminephosphotransferase activity. The only other available sequence information on this

class of proteins from mammals consists of 0.1 kb of DNA sequence from a PCR product isolated from a guinea-pig cDNA library that had 96% identity (92 of 96 bases) with the yeast *CPT1* sequence [19]. This same region possesses only 47% identity (45 of 96 bases) with the hCEPT1 described in our study. Whether the guinea-pig partial cDNA described [19] codes for an authentic mammalian cholinephosphotransferase or ethanolaminephosphotransferase awaits the isolation of a full-length cDNA and its subsequent expression and characterization.

The cloned hCEPT1 described in our study codes for a dual specificity choline/ethanolaminephosphotransferase capable of utilizing both CDP-choline and CDP-ethanolamine as phosphobase donors to a broad range of diacylglycerols, resulting in the synthesis of both PtdCho and PtdEtn. Kinetic analysis revealed that CDP-choline was preferred as the phosphobase donor. Both the cholinephosphotransferase and ethanolaminephosphotransferase activities of hCEPT1p possessed an absolute requirement for either Mg^{2+} or Mn^{2+}, with the former activity preferring Mg^{2+} and the latter preferring Mn^{2+}. This same cation preference was also observed for a partially purified choline/ethanolaminephosphotransferase activity from rat liver [33]. Two mechanisms have been proposed by which cations activate cholinephosphotransferases and ethanolaminephosphotransferases: either (1) cations bind to the CDP-aminoalcohol substrate or (2) there exists a defined cation-binding site within the enzyme [4,17]. Resolution of the mechanism of cation activation and also of the cation used *in vivo* await protein purification.

To test whether the ability of hCEPT1p to utilize both CDP-choline and CDP-ethanolamine as substrates could be recapitulated *in vivo*, hCEPT1 was expressed in an *S. cerevisiae* strain devoid of endogenous cholinephosphotransferase and ethanolaminephosphotransferase activity (HJ091, *cpt1::LEU2 ept1*$^-$) and radiolabelled choline or ethanolamine was added to exponential-phase cells in an attempt to reconstitute each pathway. Both the CDP-choline and CDP-ethanolamine pathways could be reconstituted by hCEPT1 expression, indicating that hCEPT1 has the ability to synthesize both PtdCho and PtdEtn *in vitro* and *in vivo*. In the context of mammalian cell phospholipid synthesis, it has been assumed that separate cholinephosphotransferase and ethanolaminephosphotransferase activities exist, especially in the light of the isolation of the Chinese hamster ovary (CHO) cell line with decreased ethanolaminephosphotransferase but normal cholinephosphotransferase activity [36]. The expression of hCEPT1 in this cell line cannot be performed because this line is unfortunately no longer available. The specific deficiency in ethanolaminephosphotransferase activity for this CHO cell line could be explained in several ways: (1) a structural gene distinct for ethanolaminephosphotransferase activity exists; (2) a gene capable of utilizing both CDP-choline and CDP-ethanolamine as substrates was mutated such that CDP-ethanolamine was now used less effectively as a substrate; or (3) a gene that codes for an activity that regulates the partitioning of phospholipid biosynthetic pathways was mutated such that the synthesis of PtdCho was favoured over that of PtdEtn. Whether the supply of specific diacylglycerols and CDP-aminoalcohols affects the ability of hCEPT1p to partition the PtdCho and PtdEtn biosynthetic pathways deserves further characterization. Consistent with this notion of substrate supply affecting cholinephosphotransferase or ethanolaminephosphotransferase activity *in vivo*, diacylglycerol levels have previously been demonstrated to limit the synthesis of both PtdCho [37] and PtdEtn [38] at the level of cholinephosphotransferase and ethanolaminephosphotransferase activities respectively.

The hCEPT1 transcript was detected in all cell types tested and was not enriched in any particular tissue. The ubiquitous nature of the hCEPT1 mRNA is consistent with the ability of hCEPT1 to synthesize PtdCho and PtdEtn *in vivo*.

The availability of predicted amino acid sequence data for several CDP-aminoalcohol phosphotransferases facilitated a re-examination of the theorized membrane-spanning helices of this class of enzymes from those originally predicted from the initial cloning of the first cholinephosphotransferase gene, *CPT1*, from *S. cerevisiae* [14,35]. A rearrangement of the postulated membrane spanning helices is proposed, specifically: amino acid residues corresponding to positions 180–201 within hCEPT1p were originally predicted to be exist within the solvent but are now thought to span the bilayer, and residues corresponding to positions 348–368 of hCEPT1p are no longer predicted to reside within the membrane but are now present in the solvent. Of note is an amphipathic helix spanning residues 151–168 of hCEPT1p that is also present within the corresponding region of each CDP-alcohol phosphotransferase [4,18,34]; the final two aspartic residues of the CDP-alcohol phosphotransferase catalytic motif for each enzyme lie within this amphipathic helix (Figures 6 and 7). These two aspartic residues are the catalytic residues within this motif responsible for a predicted nucleophilic attack of the hydroxy group of the hydrophobic diacylglycerol on the phosphoester bond of the water-soluble phosphobase substrate [18]. We hypothesize that the amphilicity of this helix is required to allow the interfacing of the hydrophilic CDP-choline substrate with that of the hydrophobic diacylglycerol.

A search for regulatory domains within hCEPT1p did not reveal any obvious motifs; however, several interesting regions of similarity were noted. Residues 18–34 of hCEPT1p align with residues 193–209 of argininosuccinate lyase. A previous study observed that cholinephosphotransferase became rate-limiting for PtdCho synthesis in livers of fasted hamsters [39]. The inhibitor was purified and identified as argininosuccinate. In addition, residues 252–277 of hCEPT1p align with residues 89–114 of the platelet-activating factor receptor [11–13]; the *S. cerevisiae* Cpt1p and Ept1p enzymes require activation by their products, PtdCho and PtdCho/PtdEtn respectively, in amounts indicative of a precise phospholipid-binding site within the enzymes [16,17]. Therefore this region might be capable of binding phospholipids.

In summary, this work describes the first isolation of a mammalian cDNA coding for a cholinephosphotransferase or ethanolaminephosphotransferase activity. The expressed protein is capable of utilizing both CDP-choline and CDP-ethanolamine as substrates, both *in vitro* and *in vivo*. This dual substrate specificity was unexpected and is distinct from the previously characterized role of the *S. cerevisiae* enzymes in that Cpt1p is a cholinephosphotransferase *in vitro* and *in vivo*, whereas Ept1p is a dual-specificity choline/ethanolaminephosphotransferase *in vitro* but primarily an ethanolaminephosphotransferase *in vivo* [6,40]. The isolation of the hCEPT1 will permit the precise role of this enzyme in the partitioning of phospholipid biosynthetic pathways to be defined, and a predicted but unproven role for this enzyme in the termination of diacylglycerol second messenger signals [41] to be tested.

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