Cloning of a Human cDNA for CTP-Phosphoethanolamine Cytidylyltransferase by Complementation in Vivo of a Yeast Mutant*

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CTP-phosphoethanolamine cytidylyltransferase (ET) is the enzyme that catalyzes the formation of CDP-ethanolamine in the phosphatidylethanolamine biosynthetic pathway from ethanolamine. We constructed a Saccharomyces cerevisiae mutant of which the ECT1 gene, putatively encoding ET, was disrupted. This mutant showed a growth defect on ethanolamine-containing medium and a decrease of ET activity. A cDNA clone was isolated from a human glioblastoma cDNA expression library by complementation of the yeast mutant. Introduction of this cDNA into the yeast mutant clearly restored the formation of CDP-ethanolamine and phosphatidylethanolamine in cells. ET activity in transformants was higher than that in wild-type cells. The deduced protein sequence exhibited homology with the yeast, rat, and human CTP-phosphocholine cytidylyltransferases, as well as yeast ET. The cDNA gene product was expressed as a fusion with glutathione S-transferase in Escherichia coli and shown to have ET activity. These results clearly indicate that the cDNA obtained here encodes human ET.

Phosphatidylethanolamine (PtdEtn) is a major membrane constituent of both prokaryotic and eukaryotic cells. In mammalian cells, this phospholipid can be synthesized de novo via the CDP-ethanolamine pathway, by decarboxylation of phosphatidylserine, and by the calcium-dependent exchange of ethanolamine with the base moiety of pre-existing phospholipids. Although the exact contributions of each of these pathways remain to be established, the CDP-ethanolamine pathway probably plays an important role in the synthesis of PtdEtn. CTP-phosphoethanolamine cytidylyltransferase (ET; EC 2.7.7.14) catalyzes the synthesis of CDP-ethanolamine from CTP-phosphoethanolamine cytidylyltransferase, as well as yeast ET. The cDNA gene product was expressed as a fusion with glutathione S-transferase in Escherichia coli and shown to have ET activity. These results clearly indicate that the cDNA obtained here encodes human ET.

CTP-phosphoethanolamine cytidylyltransferase (CT; EC 2.7.7.15) is a key regulatory enzyme in the CDP-choline pathway. The properties of CT have been extensively studied, and the role of CT in the regulation of phosphatidylcholine (PtdCho) biosynthesis has been well established. The sequences have been determined for several mammalian CT cDNAs (4–7) and the gene from Saccharomyces cerevisiae (8). CT exists in an inactive soluble form and an active membrane-bound form. Regulation of the translocation process by changes in the lipid composition of cellular membranes seems to be one of the major mechanisms for the control of CT activity. Phosphorylation and dephosphorylation of the enzyme are also correlated to the translocation of CT to membranes; the soluble form is highly phosphorylated, and translocation of CT to membranes is accompanied by extensive dephosphorylation. Multiple phosphorylation sites at the C terminus have been identified (9, 10). CT is thought to have a bipartite structure composed of a globular N-terminal catalytic domain and an extended C-terminal domain. Between the catalytic domain and the phosphorylation region is a sequence that is predicted to form amphipathic α-helices and to interact with the membrane bilayer of activating phospholipids (11, 12).

Unlike for CT, the protein structure and regulatory properties of ET have been studied much less extensively. ET has been purified 1,000-fold from a post-microsomal fraction of rat liver by Sundler (13). van Golde et al. recently purified ET to homogeneity from rat liver (14). The purified protein has a molecular weight of about 50,000 and appears to be a dimer. ET has long been tacitly assumed to be regulated like CT. However, there is accumulating evidence that the control mechanisms for the ET and CT activities are different (15).

Only sequence information for the yeast S. cerevisiae gene putatively encoding ET is available. Yeast cells possess phospholipid-synthetic pathways quite similar to those found in other eukaryotic cells. Yeast gene ECT1, originally known as MUQ1 (16), encodes a protein similar to CTs of yeast and other species and, therefore, is thought to encode ET. Here, we report the isolation and characterization of a human cDNA encoding ET by genetic complementation of a yeast mutant defective in ECT1.

EXPERIMENTAL PROCEDURES

Strain, Media, and Growth Conditions—Saccharomyces cerevisiae DS15–3 (MATa isoleu2 his3) was used as the wild-type strain and as the recipient for ECT1 disruption. DS15–3 is a segregant of the diploid strain, DS15 (17). The isoleu2 mutant is a conditional ethanolamine auxotroph whose growth is inhibited by high concentrations of inositol, and the growth defect is suppressed by the addition of ethanolamine to the media.

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culture medium (18). Yeast cells were cultured in either YPD or synthetic minimal medium at 30 °C. The compositions of the YPD and inositol-free minimal media were reported previously (18). The composition of nitrogen-free minimal medium (M-N) is given in Ref. 19.

Inositol, L-leucine and L-histidine were each added to the culture media at the A/3 level. DNA was extracted from the culture media at the concentration of 10 μg/ml.

Amplification of ECT1—The yeast ECT1 gene was amplified from chromosomal DNA by the polymerase chain reaction (PCR). The PCR primers used were 5'-CGGATCCTCTAGATGACGGTAAACTTA-3' (forward primer) and 5'-CCGGCAATACCAACCCGTTCTCTAGATGACGGTAACTTA-3' (reverse primer). The complementary oligonucleotides contained additional sequences (underlined), so there was a BamHI restriction site at the 5' end and a SaI site at the 3' end of the PCR products. An about 1-kbp PCR product was digested with BamHI and SaI, purified by gel electrophoresis, and then inserted between the BamHI and SaI sites of pUC18 to yield pUC-ECT. By comparing the restriction maps of the isolated clone and the reported one, partial sequencing of the cloned fragment, plasmid pUC-ECT was confirmed to contain the yeast ECT1 gene.

Construction of an ECT1-disrupted Strain—The 0.4-kbp EcoRV/XhoI fragment of pUC-ECT located within the ECT1 coding frame was replaced with a 1.3-kbp HincII/Xhol fragment containing yeast HIS3 to yield pUC-ect:HIS3. The 1.9-kbp SphI/SalI fragment of this plasmid was used for the transformation of DS15-3 H- cells were used. One disruptive strain, of which the gene disruption was confirmed by Southern blot analysis, was designated as NA9 and used for the isolation of human cDNA.

cDNA Cloning—A human cDNA library made from glioblastoma cells was constructed on a yeast multicycop vector, pDANS, which contains the yeast ADH1 promoter, the following small part of the coding region, and the terminator (20). Inserted cDNAs are designed to be expressed in yeast cells as a fusion protein with ADH1 N-terminal amino acids. NA9 was transformed with the cDNA library, and the colonies that grew on a minimal medium supplemented with inositol and ethanolamine were selected. Colonies that grew on a medium supplemented with inositol alone were selected. Plasmids were recovered from the transformants and used for Escherichia coli transformation.

DNA Sequencing and Sequence Analysis—DNA was sequenced manually using the dideoxynucleotide chain termination method (21) with Klenow fragment (Takara Shuzo) and [32P]dCTP (Amersham Corp.) on the M13 phase vector. The primers were either universal or a series of oligonucleotides synthesized for the partial cDNA sequence. Amino acid sequence similarities were found using the BLAST program, and the translation of nucleotide sequences in the GenBank and EMBL data bases. Alignments were further improved by eye. Only amino acid identities were considered.

Transcript Analysis—An adult human multisissue Northern blot (Clontech) was hybridized with a random primed (Takara Shuzo) [32P]-labeled entire cDNA fragment as a probe. Hybridization was carried out at 42 °C overnight in a hybridization solution containing 50% formamide, 50 mM sodium phosphate (pH 6.5), 5 × SSC (1 × SSC: 0.15 mM NaCl, 15 mM sodium citrate, pH 7.0), 1 × Denhardt’s (0.1% each of bovine serum albumin, polyvinylpyrrolidone, and Ficoll), and 0.1% SDS. The filter was washed twice at room temperature with 2 × SSC containing 0.1% SDS for 10 min, and then twice with 0.1 × SSC containing 0.1% SDS at 50 °C for 15 min.

Metabolite Analysis of Radiolabeled Ethanolamine—Exponentially growing yeast cells were harvested by centrifugation, washed with M-N medium, and then resuspended in the same medium. To 1 ml of culture of each strain was added 7.4 kBq of [1,2-14C]ethanolamine and 0.4 μmol of ethanolamine, and then the mixture was incubated at 30 °C. At the indicated times, 5 ml of 10% trichloroacetic acid was added to the culture. The mixture was then filtered through GFC glass-microfiber paper (Whatman). The filter paper was washed three times with 5 ml of 10% trichloroacetic acid and dried, and then radioactivity was counted after treatment with Scintilamine-OH (Dojindo) as described previously (21). The uptake of the labeled precursors into cells during the above incubation was determined by counting the radioactivity in cells collected by filtration through GFC glass-microfiber paper as described previously (22).

For the lipid analysis, cells were cultured under the specified conditions, collected by centrifugation, and then suspended in M-N medium. To 2 ml of cell suspension was added [1,2-14C]ethanolamine or [methyl-14C]choline (37 kBq each). After the incubation at 30 °C for 60 min, cells were collected by centrifugation, suspended in 100 μl of methanol, and then sonicated. To the sonicated sample was added 200 μl of chloroform to extract the lipids. The extract was washed once with 300 μl of saline, twice with 300 μl of methanol-saline (1:1, v/v), and then evaporated. Lipids were dissolved in 50 μl of chloroform and chromatographed on a Silica Gel 60 plate (Merck) with chloroform-methanol-acetic acid-formic acid-water (35:15:6:2.1, by volume) as a developing solvent. Radioactive materials were detected by autoradiography.

Construction of a GST Fusion Protein and Expression in E. coli—A Smal/EcoRI fragment excised from the cDNA clone was ligated between the Smal and EcoRI sites of pGEX-2T (Pharmacia Biotech Inc.). The plasmid, pGEX-hECT, thus obtained was used for expression in E. coli. Briefly, overnight cultures of E. coli JM103 harboring the pGEX-hECT plasmid were diluted in fresh medium and then grown for another 2 h at 37 °C. Protein expression was induced with 1 mM isopropyl-1-thio-β- galactopyranoside (IPTG), and after a further 3 h of growth, the cells were pelleted and resuspended in 10 mM Tris-Cl buffer (pH 7.5) containing 1 mM EDTA and 20% glycerol. The cells were then lysed by sonication and centrifuged at 8,000 × g for 10 min. The supernatant was used for the purification of the fusion enzyme. A part of the IPTG-treated and untreated cells, respectively, was used for protein analysis. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) of total E. coli proteins was performed in 10% polyacrylamide mini-slab gels, essentially as described by Laemmli (23). Molecular weight references were purchased from Sigma. Proteins were stained with Coomassie Brilliant Blue using a Quick-CBB kit (Wako).

ET activity of fusion protein was determined in a reaction mixture comprising 20 mM Tris-Cl (pH 7.5), 10 mM MgCl2, 2 mM CTP, 5 mM dithiothreitol, [1,2-14C]phosphoethanolamine, and E. coli cell extract, in a total volume of 25 μl. After 30 min of incubation at 30 °C, the reaction was stopped by heating at 100 °C for 5 min. The reaction product was separated by thin-layer chromatography on a Silica Gel 60 plate as described above. Protein determination was carried out with BCA protein assay reagent (Pierce).

ET Activity—Yeast cells were cultured in a minimal medium on a Bio-Shaker BR-30 (Taieto) to the early stationary phase at 30 °C, harvested, and disrupted by vortexing vigorously with glass beads (diameter, 0.3 mm) in 10 mM Tris-Cl containing 1 mM EDTA and 20% glycerol for 5 min. Disrupted cells were centrifuged at 4,000 × g for 10 min, and the supernatant from these cells was used for the determination of ET and CT activities as described previously (22) except that the total volume was reduced to 50 μl. All enzyme reactions were carried out at 25 °C for 10 min or 20 min.

Materials—Restriction endonucleases and other nucleic acid-modifying enzymes were purchased from Takara Shuzo. Taq DNA polymerase was obtained from Boehringer Mannheim. [α-32P]dCTP (110 TBq/ mmol), [methyl-14C]choline (2.04 GBq/mmol), and [methyl-14C]phosphocholine (2.07 GBq/mmol) were obtained from Amersham. [1,2-14C]Ethanolamine (170 MBq/mmol) was from DuPont NEN. CTP was from Yamasa. Choline chloride, ethanolamine, phosphocholine, and phosphoethanolamine were from Tokyo Kasei. Other chemicals were from Wako Chemicals. [1,2-14C]Phosphoethanolamine was prepared from [1,2-14C]ethanolamine through the action of yeast choline kinase (24) and purified as described by Sundler (25).

RESULTS

Isolation of Human cDNAs by Complementation in Vivo of a Yeast Mutant—Based on the structural similarity, the S. cerevisiae ECT1 gene was thought to encode ET (16). To confirm this, we constructed an yeast strain of which the ECT1 gene was disrupted. Although mutants carrying the ett1 mutation alone could exhibit no growth phenotype, the combination of the ett1 and ise mutation allowed us to detect the phenotype of the ett1 mutation. The ise mutation is a conditional choline-ethanolamine-auxotrophic mutant of which the growth is inhibited by high concentrations of inositol (18). As was expected, an ett1 ise double mutant, NA9, we constructed did not grow on inositol- and ethanolamine-containing media, although it grew normally on inositol- and choline-containing media or on inositol- and ethanolamine-containing media.
The cDNA gene product was found to be similar to several cytidylyltransferases and found to be most similar (36% identity) to the Arabidopsis thaliana protein kinase, which has already been isolated (26). On the other hand, the sequence similarity revealed that plasmid pH4 contains the cDNA for human choline kinase, which has already been isolated (26). On the other hand, plasmid pS4a was found to have a new cDNA. We therefore sequenced the entire region of the new cDNA.

**Nucleotide and Deduced Amino Acid Sequences**—A restriction map of the cDNA insert of pH4 is shown in Fig. 2A. The nucleotide sequence of the cDNA was determined on both strands. The cDNA insert consisted of a total of 1,856 bases, with a single long open reading frame (ORF) starting with an ATG codon at position 67 and ending at position 1,236 (Fig. 2B). Although it was not known whether translation started at the first methionine shown in Fig. 2B or at a further upstream one, we predicted that the first methionine shown in Fig. 2B is the likely translation start site, as the sequence flanking it conforms to the consensus sequence for translation initiation proposed by Kozak (27), (A/G)XAGG(A/G)X(C/T). This was supported by sequence comparison with the yeast ET (see below). The ORF encoded a protein of 389 amino acids with a calculated molecular mass of 43.8 kDa. As was expected, the translation start site of the cDNA obtained here is for human ET. We describe below biochemical evidence demonstrating that this is indeed the case.

**Northern Analysis**—Northern analysis was carried out with a variety of adult human tissues to search for a cDNA probe that hybridizes to a variety of tissues. The probe detected a major transcript of approximately 2.1 kb in a variety of adult human tissues. For this study the entire nucleotide sequence of the human cDNA was determined. Fig. 2C shows the hybridization of cDNA probes to human adult poly(A)-containing RNA from various adult human tissues. As shown in Fig. 2C, a major transcript of approximately 2.1 kb was detected in liver, heart, and skeletal muscle tissues. The size of the transcript is consistent with that of the cDNA obtained. The transcript is very abundant in liver, heart, and skeletal muscle tissues.

**Lipid Synthesis via the CDP-ethanolamine Pathway**—To confirm that plasmid pS4a contains the cDNA for human ET, between the cDNA gene product and CTs from several species, including from humans, was limited to their N-terminal halves. Limited sequence similarity was also observed at the C termini of human and yeast ET, and yeast CT, but not at the C terminus of human CT (Fig. 3C). These results strongly suggested that the cDNA obtained here is for human ET. We describe below biochemical evidence demonstrating that this is indeed the case.

**FIG. 1.** Suppression of the growth defect of yeast cells by human cDNAs. *See et al.* double mutant NA9 was transformed with the indicated plasmids, and then its growth phenotype was examined on a minimal medium supplemented with or without inositol plus ethanolamine. Plasmid pADANS is the multicycopy vector used for the construction of the human cDNA library.

**FIG. 2.** Nucleotide and deduced amino acid sequences of the human cDNA. A, restriction map of the cDNA in plasmid pH4. The hatched box represents the coding region. The restriction sites are: EcoRI, HindIII, PstI, PvuII, SacI, SmaI, SphI, EcoRI, HindIII, and NotI are cloning sites of vector pADANS. B, nucleotide sequence of the cDNA and the deduced amino acid sequence, which is shown below the nucleotide sequence in the one-letter code. Residue numbers for nucleotides and amino acids are shown on the right. The putative initiation codon is indicated by the underline. The asterisk denotes the termination codon. The double-underline indicates a putative polyadenylation signal.
we examined whether or not the cDNA could restore the activity of the CDP-ethanolamine pathway in the yeast strain. The flux of ethanolamine through the ET step was analyzed by determining the incorporation of [14C]ethanolamine into PtdEtn. The overall rate of PtdEtn synthesis was determined by measuring the radioactivity in lipids. As shown in Fig. 5, the incorporation of radioactivity in the \textit{ect1} mutant was very low. On the other hand, the incorporation of radioactivity into lipids in the mutant strain harboring plasmid pS4a had recovered to 81% (at 60 min) of that in the wild type. It was also shown that the introduction of the human cDNA for choline kinase (plasmid pH4) restored the incorporation of radiolabeled ethanolamine into lipids, although the extent was slightly low (43% of that in the wild type at 60 min).

We next analyzed the radioactive lipids derived from [14C]-labeled ethanolamine and choline. Cells were cultured with labeled compounds for 60 min and lipids were extracted and analyzed by thin-layer chromatography. As shown in Fig. 6, the amount of labeled PtdEtn and PtdCho derived from [14C]ethanolamine was very low in the mutant cells (lane 5) when compared with those in wild-type cells (lane 4). Introduction of human cDNA into the yeast mutant clearly restored the synthesis of PtdEtn and PtdCho from [14C]ethanolamine (lane 6). In contrast, there were no significant differences in the amount of labeled PtdCho derived from [14C]choline between these strains (lanes 1–3).

We next examined the incorporation of [14C]ethanolamine into the intermediates of the CDP-ethanolamine pathway. The \textit{ect1} mutant harboring vector plasmid pADANS and plasmid pS4a, as well as the wild-type strain harboring pADANS were grown at 30 °C in the presence of [14C]ethanolamine for 60 min. Trichloroacetic acid-soluble materials were analyzed by thin-layer chromatography. As shown in Fig. 7, mutant cells accumulated much higher levels of radioactive phosphoethanolamine than wild-type cells. Conversely, the level of radioactive CDP-ethanolamine was very low in the mutant. Introduction of
the human cDNA into the yeast mutant clearly restored the level of CDP-ethanolamine. These results strongly indicate that the cDNA product has ET activity. Further evidence supporting this conclusion was obtained by assaying the ET activity of the cDNA product.

**ET and CT Activities—** ET and CT activities in the transformant were determined and compared with those in the mutant and wild-type cells. As shown in Table I, *ect1* mutant strain, NA9 having a vector alone, had a very low ET activity, when compared with wild-type strain, DS15–3. On the other hand, the introduction of human cDNA on a multicopy vector into the mutant cell clearly restored the ET activity. The activity was higher than that in wild-type cells. This might be the results of gene dosage effect. CT activity was measured as a control, and we found no significant differences in the enzyme activities between these strains.

**Expression of Human ET in E. coli—** To determine the ET activity of the cDNA product, we expressed its protein product fused with GST in *E. coli*. A *Sma*I/*Eco*RI cDNA fragment was subcloned between the *Sma*I and *Eco*RI sites of expression vector pGEX-2T. The plasmid, pGEX-hECT, thus obtained resulted in an in-frame fusion between the N-terminal GST coding region and codons 18–390 of the ET coding region (including the stop codon). The construct was transformed into *E. coli*, and expression of the fusion protein was induced with IPTG. The production of the fusion protein was analyzed by SDS-PAGE. The IPTG-dependent induction of a large amount of the fusion protein was detected (data not shown). The molecular mass of the fusion protein appeared to be about 65 kDa, this being consistent with the calculated value.

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We next determined the ET activity using [14C]phosphoethanolamine as the substrate and an *E. coli* cell extract containing the fusion protein as the enzyme source. Reaction products were analyzed by thin-layer chromatography and located by

**Fig. 4. Tissue-specific expression of human ET mRNA revealed by Northern blot analysis.** A, a human multitissue blot containing poly(A)⁺ RNA (Clontech) was probed with a radiolabeled cDNA probe and then autoradiographed. The positions of RNA size markers are indicated on the left. B, the same blot was rehybridized with a manufacturer-supplied β-actin DNA as a probe.

**Fig. 5. Incorporation of [14C]ethanolamine into PtdEtn in the wild-type strain, the *ect1* mutant NA9, and the mutants harboring human cDNAs.** To 1 ml of a culture of each strain was added 7.4 kBq of [14C]ethanolamine, and then the mixture was incubated at 30 °C. At the indicated times, the incorporation of radioactivity into lipids was determined as described under “Experimental Procedures.” A₅₅₀ refers to the absorbance at 550 nm. □, wild type with pADANS; ●, mutant with pADANS; ○, mutant with pH4; ●, mutant with pS4a. The total radioactivity taken up into cells at 60 min was 3.3 × 10⁴, 3.3 × 10⁴, 5.0 × 10⁴, and 3.5 × 10⁴ dpm, for wild type with pADANS, mutant with pADANS, mutant with pH4, and mutant with pS4a, respectively.

**Fig. 6. Analysis of radiolabeled PtdEtn and PtdCho.** Yeast cells were incubated with 37 kBq of [14C]choline (lanes 1–3) or 37 kBq of [14C]ethanolamine (lanes 4–6) at 30 °C for 60 min. Lipids were extracted and analyzed by thin-layer chromatography as described under “Experimental Procedures.” Lanes 1 and 4, wild type; lanes 2 and 5, mutant with pADANS; lanes 3 and 6, mutant with pS4a.

**Fig. 7. Analysis of the intermediates of the CDP-ethanolamine pathway.** Yeast cells were incubated at 30 °C with 37 kBq of [14C]ethanolamine for 60 min. Radiolabeled water-soluble metabolites were analyzed by thin-layer chromatography as described under “Experimental Procedures.”}

## Table I

<table>
<thead>
<tr>
<th>Strain</th>
<th>Plasmid</th>
<th>ET Specific activity</th>
<th>CT Specific activity</th>
</tr>
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<tbody>
<tr>
<td>DS15–3</td>
<td>None</td>
<td>1.13 ± 0.26</td>
<td>0.87 ± 0.35</td>
</tr>
<tr>
<td>NA9</td>
<td>pADANS</td>
<td>0.07 ± 0.05</td>
<td>1.38 ± 0.03</td>
</tr>
<tr>
<td>NA9</td>
<td>pS4a</td>
<td>2.68 ± 0.82</td>
<td>1.65 ± 0.40</td>
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</tbody>
</table>

* Specific activity data are the averages ± standard deviations from four independent experiments.
The yeast kinase from the same library as used here by complementing structural and functional analyses of ETs are required. Further studies involving the cDNA consists of 389 amino acids with a molecular mass of approximately 49.5 kDa (11, 12).

We describe here the first isolation and characterization of the cDNA for ET from mammalian cells. The size of the mRNA for ET, as revealed by Northern blot analysis, was almost the same as that of the cDNA. The transcript is very abundant in liver, heart, and skeletal muscle tissues. The high level of expression in liver cells agrees with the fact that liver cells have high ET activity (2). The deduced polypeptide encoded by the cDNA consists of 389 amino acids with a molecular mass of about 43.8 kDa. This value is slightly less than that of ET purified from rat liver, i.e. approximately 49.5 kDa (11, 12).

The predicted amino acid sequence of the putative encoded protein shows a high degree of similarity to the yeast ET throughout the entire region. The putative human protein, however, is longer than the yeast protein in both the N- and C-terminal regions, especially at the C-terminal region. The two proteins are functionally similar. Indeed, the introduction of the human cDNA, even in a single copy, restored the growth defect of the yeast ect1 mutant (data not shown). An additional striking structural feature is that human and yeast ETs have a large repetitive sequence in their N-terminal and C-terminal halves, suggesting that the two halves are generated through a gene duplication event. The regions of greatest identity in the two halves of ETs are also homologous to the sequences in the N-terminal half of CTs. Based on the high degree of homology between the N-terminal domain of rat CT, a similarly located region in yeast CT, and a Bacillus subtilis glycero-phosphatidylcholine synthase (4, S, 28), it has been proposed that this region bears the active site of these cytidylyltransferases. This was confirmed by that the catalytic activity of a truncated form of rat CT, comprising residues 1–236, was quite similar to that of the wild-type CT (29). Therefore, human and yeast ETs might have two catalytic domains, and this might reflect the substrate specificity of the enzyme. Further studies involving structural and functional analyses of ETs are required.

In a previous study, we cloned the human cDNA for choline kinase from the same library as used here by complementing the yeast cki1 mutation (26). Choline kinase catalyzes the formation of phosphoethanolamine, which is one of the substrates for ET. As shown in Fig. 1, we again obtained the human choline kinase cDNA as a suppressor for the yeast ect1 mutation. It was also shown that the human choline kinase cDNA restored the defect of PtdEtE synthesis via CDP-ethanolamine when introduced into the yeast ect1 mutant (Fig. 5). These results strongly suggest that the introduction of multicopies of the choline kinase gene might result in overproduction of phosphoethanolamine, and the accumulation of phosphoethanolamine could bypass the defect of ect1 through the action of CT. Indeed, this is the case because we found that multicopies of the yeast CKI1 or CCT1 gene can also suppress the growth defect of the ect1 mutant (data not shown). Although phosphoethanolamine is a poor substrate for CT (22, 30), elevation of the enzyme activity of CT on introducing multicopies of CCT1 into cells could compensate for the defect of ET activity.

Based on the analogy between the CDP-choline and CDP-ethanolamine pathways, it has long been thought that ET would be regulated in a similar manner to CT. However, more recent studies summarized by Tijburg et al. (15) strongly suggest that, at least in liver, the CDP-choline and CDP-ethanolamine routes are subject to independent regulation. Further work on thorough comparison of the two cytidylyltransferases and the significance of structural differences that could be relevant for independent regulation of these enzymes are required. The cDNA and the information on the primary structure for ET obtained here will allow us to develop methods for studying the structural and functional relationship between ET and CT, as well as the regulatory mechanism for ET.

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