Structure and expression of CTP:phosphocholine cytidylyltransferase gene from *Arabidopsis thaliana*

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Phosphatidylcholine is the major phospholipid component of eukaryotic membranes. The nucleotide pathway of *de novo* synthesis of phosphatidylcholine consists of three consecutive reactions. CTP:phosphocholine cytidylyltransferase (EC 2.7.7.15) catalyzes the second reaction, the conversion of phosphocholine to CDP-choline, which is generally believed to be the key regulatory step of the pathway. The *A. thaliana* cytidylyltransferase cDNA is 1447 bp long and contains an open reading frame of 993 bp coding for a protein of 331 amino acids. The deduced structure of the enzyme was composed of three main regions; the catalytic domain in the N-terminal half, the hydrophilic C-terminal region and the amphipathic domain in the middle. In the region between the catalytic domain and the C-terminal region, there was an amphipathic α-helical domain, which was believed to bind the membrane surface in the active formation. The identity of cytidylyltransferase cDNA was verified by successful transformation of an yeast mutant defective in the enzyme activity. This was further confirmed by *in vivo* analysis of the enzyme reaction product after labelling the yeast transformants with radioactive phosphocholine.

A genomic clone which includes the cytidylyltransferase open reading frame and its 5'- and 3'- flanking non-coding regions has also been isolated from *Arabidopsis thaliana*. The cytidylyltransferase gene is approximately 3.0 kb in length and contains 8 exons interrupted by 7 introns, which range from 74 to 626 nucleotides. In 5'-flanking region there are two sequences related to a cold-responsive element found in the cold-inducible promoter of the *A. thaliana cor15a* gene, plus one gibberellin-response element. The results from reverse transcriptase-PCR indicate that expression of *A. thaliana* cytidylyltransferase was regulated by temperature.