Characterization of UDP-glucose:ceramide glucosyltransferases from different organisms

M. Leipelt*, D. C. Warnecke*, B. Hube*, U. Zähringer† and E. Heinz*

*Institut für Allgemeine Botanik, Universität Hamburg, Ohnhorststraße 18, D-22609 Hamburg, Germany, and
†Forschungszentrum Borstel, Parkallee 22, D-23845 Borstel, Germany

Abstract
Cerebrosides are typical membrane lipids of many organisms. They occur in plants, fungi, animals, humans and some prokaryotes. Almost all of our knowledge on the physiological functions of cerebrosides results from experimental data obtained with mammalian cells. However, very little is known about the roles played by these lipids in plants and fungi. To initiate such investigations we have cloned and characterized a ceramide glucosyltransferase from the yeast Candida albicans. Functional expression of this gene in Saccharomyces cerevisiae led to the accumulation of new glycolipids which were not present in wild-type baker’s yeast. They were identified by MS and NMR spectroscopy as β-D-glucopyranosyl ceramides. The ceramide moieties of these cerebrosides comprised phytosphinganine and mainly long-chain (C2& α-hydroxy fatty acids in amide linkage. We also generated a ceramide glucosyltransferase-knock-out strain of C. albicans which was devoid of cerebrosides. The viability of this mutant showed that for this organism glucosyl ceramides are not essential for vegetative growth on complete or minimal media. In addition, we have cloned and functionally expressed one of the three putative glucosylceramide synthases from Caenorhabditis elegans, as well as a corresponding enzyme from Pichia pastoris.

Introduction
Glycosphingolipids (GSLs), amphipathic compounds consisting of sugar and ceramide moieties, are ubiquitous components of the plasma membranes of eukaryotic cells, whereas in prokaryotic cells GSLs are extremely rare. GSLs show heterogeneity not only in their sugar chains but also in their ceramide moieties. These GSLs are believed to play important roles in a variety of cellular processes such as cell recognition, growth, development and differentiation [1]. Ceramide glucosylation is also important in controlling the intracellular level of ceramide, which is regarded as functioning as a second messenger. In the pathway of GSL synthesis, the first step is transfer of glucose or galactose to a ceramide to produce glucosylceramide (GlcCer) or galactosylceramide, respectively. These reactions are catalysed by a UDP-glucose:ceramide glucosyltransferase and a UDP-galactose:ceramide galactosyltransferase, respectively [2]. Since over 400 different glycolipids are derived from GlcCer, ceramide glucosyltransferase (GlcT) is an extremely important glycosylation enzyme. In 1996, a cDNA for human GlcT-1 was isolated by expression cloning using mouse cells deficient in GlcT-1 activity [3]. GlcT-1 is a typical type-III glycosyltransferase which possesses the transmembrane domain at its N-terminus. The catalytic domain of GlcT-1 is located on the cytosolic side of the Golgi membrane.

Database searches reveal similar sequences in mouse, rat, Caenorhabditis elegans, cyanobacteria, Drosophila melanogaster and yeasts, indicating that ceramide glucosylation is of wide biological importance.

To gain more insight into the role of ceramide glucosylation in yeast and fungi, it is necessary to isolate and characterize the corresponding GlcTs from these organisms. Here we describe the identification and characterization of UDP-glucose:ceramide glucosyltransferase from Candida albicans. We expect that genetic manipulation of this gene will facilitate the elucidation of ceramide glycoside function in C. albicans and other yeasts.

Materials and methods
We searched in the C. albicans genome database (Stanford) for sequences related to the human UDP-glucose:ceramide glucosyltransferase gene. One open reading frame (ORF) of unknown function showed significant similarity with the human GlcT-1 gene. For expression in yeast, PCR
primers were designed to amplify the predicted ORF of interest with genomic DNA of *C. albicans* as a template. The putative UDP-glucose:ceramide glucosyltransferase sequence was cloned by PCR into the pVT102-U vector downstream of the ADH1 promoter. This construct was introduced into *Saccharomyces cerevisiae* strain 334 by a modified poly(ethylene glycol) (PEG)/lithium acetate method [4]. Growth of transgenic yeasts and lipid extraction as well as the derivatization of the glycolipid for analysis by TLC, MS and NMR analysis were performed as described earlier [5,6].

We also generated a *C. albicans* mutant strain deficient in UDP-glucose:ceramide glucosyltransferase by targeted gene disruption using a *hisG*::URA3::*hisG* cassette as described earlier [7].

**Results and discussion**

A BLAST database search with the amino acid sequence of human UDP-glucose:ceramide glucosyltransferase (EC 2.4.1.80) [3] showed significant similarity between this sequence and the deduced amino acid sequence of an ORF of unknown function from *C. albicans*. According to BLAST [8] the similarity of the complete amino acid sequence deduced from the *Candida* sequence with UDP-glucose:ceramide glucosyltransferase was 40% at an identity of 22%. The entire gene was amplified by PCR using specific primers and cloned into pBluescript for sequencing.

For functional identification, the ORF was expressed in *S. cerevisiae*. *S. cerevisiae* is suitable for analyses of genes involved in GlcCer synthesis because it does not contain GlcCer, whereas its precursor is present. For expression we used a system in which the genes are controlled by the constitutive ADH1 promoter. Total lipid extracts of the transformed and the corresponding untransformed *S. cerevisiae* cells were prepared and analysed by TLC. These transformed cells produced a new glycolipid that was not present in the wild-type yeast. It was detected by sugar-specific reagents, but it did not react with a phosphate-specific spray reagent. The glycolipid band cochromatographed with an authentic cerebroside standard. The new glycolipid was purified by preparative TLC and peracylated. The peracetylated glycolipid has been identified by MS and NMR spectroscopy as β-ν-glucopyranosyl ceramide.

By an analogous procedure, we have also cloned one of the three GlcCer synthases annotated in the *C. elegans* genome, from which another one had already been identified [9]. Expression of this gene in baker’s yeast resulted in the accumulation of cerebrosides, which after isolation were identified as described above by spectroscopic methods. A similar approach led to the identification of a gene from *Pichia pastoris*, which after functional expression in yeast suggests that we also have cloned a GlcCer synthase from this organism.

To gain insight into the role of ceramide glucosylation in fungi, we generated a knock-out mutant of *C. albicans* deficient in GlcT. The total lipid extracts of this null mutant did not contain detectable amounts of cerebrosides. The viability of the mutant strain shows that for this organism glucosyl ceramides are not essential for vegetative growth on complete or minimal media. These data prove that the cloned ORF from *C. albicans* indeed encodes a GlcT.

The identification of new yeast sequences encoding GlcTs will also encourage new attempts for understanding ceramide glucosylation in plants.

**References**


Received 30 June 2000