Sphingolipids in Health and Disease

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Our laboratory works on sphingolipids (SLs), important lipid components of eukaryotic cell membranes. Three major projects are currently underway. In the first, we are studying SL biosynthesis, and in particular, a protein family that is responsible for the synthesis of ceramide, an important lipid second messenger. In the second, we are attempting to delineate the molecular mechanisms by which SL accumulation in inherited metabolic disorders, such as Gaucher disease, Tay-Sachs (Sandhoff) and Niemann-Pick disease, causes cell dysfunction, and hence disease. In the third, we are working on structure-function relationships of the enzyme, glucocerebrosidase, which is given to Gaucher disease patients in enzyme replacement therapy (ERT).

In the first project, we are studying how ceramide synthesis is regulated by the Ceramide Synthase (CerS) protein family (Fig. 1). Ceramide consists of a sphingoid long chain base to which a fatty acid is attached, and we have demonstrated that each of the CerS synthesizes ceramide with a highly selective fatty acid composition. Thus CerS1 synthesizes ceramide containing a C18-fatty acid, CerS5 synthesizes ceramide with a C16-fatty acid, and CerS2 synthesizes ceramide with longer chain fatty acids, i.e. C22-C26. CerS are part of a larger family of TLC domain-containing proteins (Fig. 2), and the function of most of the proteins in the TLC family are still unknown.

The reason that mammals have multiple CerS genes, whereas most of the other enzymes in the SL biosynthetic pathway exist in only one or two isoforms, is not known, but implies an important role for ceramides containing specific fatty acids in cell physiology, particularly in signaling pathways, in which specific ceramides containing a restricted set of fatty acids are generated in response to certain stimuli. However, very little is known about the relationship between levels of specific ceramides and the expression of CerS genes in different cells and tissues. With a view to understanding this relationship, we have recently began to analyze the distribution of CerS genes by qRT-PCR, which revealed that CerS2 mRNA is found at the highest level of all CerS, and has the broadest tissue distribution. Moreover, the activity of CerS2 can be regulated by another bioactive sphingolipid, sphingosine 1-phosphate (S1P), via interaction of S1P with two residues that are part of an S1P receptor-like motif found only in CerS2. Thus, CerS2 may be regulated post-translationally in a unique manner, and study of its genomic organization also suggest some unique regulatory features. CerS2 has a compact gene size, a low number of introns, short 5′- and 3′-UTRs, with a large percentage of surrounding chromosomal sequence containing CpG and Alu elements, and contains a low percentage of LINE-1s. Further, CerS2 is located within chromosomal regions which are replicated early within the cell cycle. These genomic features are characteristic of a 'housekeeping' gene; indeed, predictive analysis performed using multiple parameters supports the possibility that the CerS2 gene is a genuine house-keeping gene. Interestingly, no other CerS genes display these characteristics. We are currently attempting to understand the modes of regulation of CerS2, and the role of CerS2 in cell physiology.

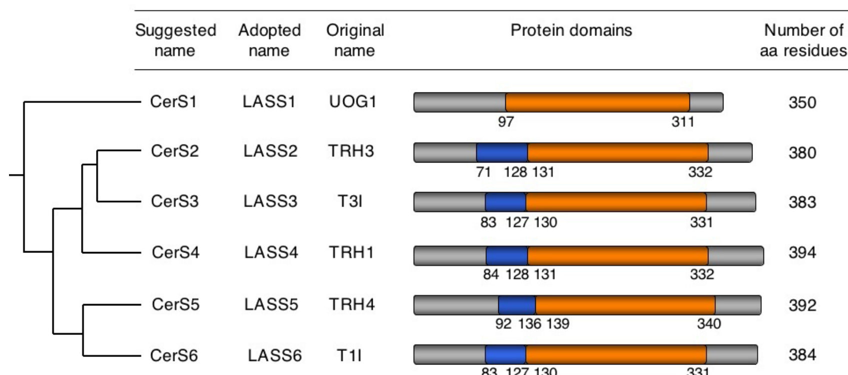

Fig. 1 *Phenogram, predicted domains, and terminology of human CerS proteins. The phenogram shows the relationship between CerS genes. Protein domains are as predicted by Prosite; the Homeobox domain is in blue, and the TLC domain in orange. Numbers represent the amino acid residues at the beginning and end of these regions. From Pewzner-Jung, Y. et al, 2006.*

In the second project, we are studying the pathophysiological mechanisms of SL storage diseases, which are largely unknown. In a mouse model of Gaucher disease (Gba mice), in which glucosylceramide (GlcCer) accumulates, we demonstrated that there is a significant increase in the rate of Ca^{2+}-release from the endoplasmic reticulum (ER) via the ryanodine receptor (RyaR), resulting in elevated cytosolic Ca^{2+} levels which leads to enhanced sensitivity to agents that induce cell death. Cytosolic Ca^{2+}-levels are also elevated in a mouse model (the Hexb mouse) of Sandhoff disease (a form of Tay-Sachs disease), but in contrast to Gba neurons, this is caused by changes in the rate of Ca^{2+}-uptake into the ER via the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA), rather than by changes in the rate of Ca^{2+}-release. In the case of Sandhoff disease, we have explored the precise structural requirements of GM2 that are required to inhibit SERCA activity, and have shown that

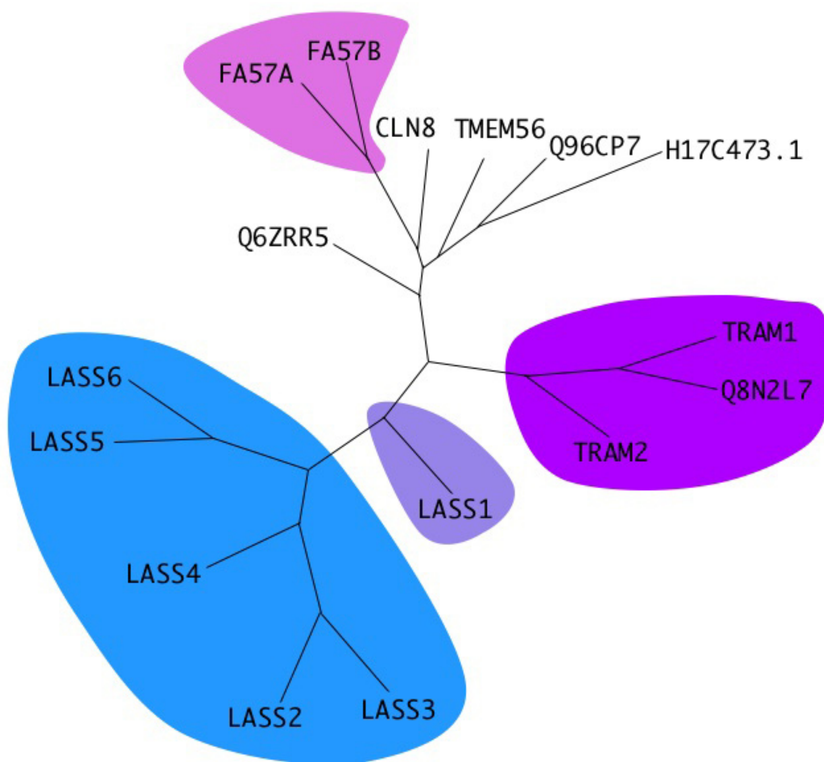

Fig. 2 *Phylogenetic tree of the 16 known human TLC domain-containing proteins. Sequences were taken from Swiss-Prot with the exception of H17C473.1, which does not have a full length mRNA in human, and is based on a gene model from Ecgeneand which closely matches the mouse cDNA available for the gene. From Pewzner-Jung, Y. et al, 2006.*

an exposed carboxyl group on the ganglioside sialic acid residue is required for inhibition. Finally, in Niemann-Pick A disease, we have also demonstrated changes in Ca^{2+}-homeostasis, but in this case the changes are caused by degeneration of a specific population of cerebellar neurons enriched in SERCA2, consistent with the previously observed loss of Purkinje cells in the cerebellum of a mouse model of Niemann-Pick A disease. Together, our data suggest a biochemical mechanism to explain some of the neuropathophysiology in SL storage diseases.

Finally, together with Israel Silman and Joel Sussman, we are working on structure-function relationships of acid beta-glucosidase (GlcCerase), the enzyme used in enzyme replacement therapy in Gaucher disease. Since we originally solved the X-ray structure of Cerezyme®, the product produced by Genzyme, we have gone on to solve the structure of Cerezyme® conjugated to chemical inhibitors, which has given insight into the mode of action of the inhibitors in chaperone-mediated therapy. We have also solved the structure of GlcCerase produced using a plant cell system, in collaboration with Protalix Biotherapeutics. The plant-produced enzyme displays a level of biological activity similar to that of Cerezyme®, as well as a highly homologous three dimensional structure (Fig. 3). The plant cell enzyme has also been co-crystallized with small chemical inhibitors, which lead to some unexpected findings relating to the involvement of loops adjacent to the active site in the catalytic process, the reaction mechanism, and the role of a conserved water molecule located in a solvent cavity adjacent to the active site. Together, these results have significance for understanding the mechanism of action of GlcCerase, and the mode of GlcCerase chaperoning by imino sugars, and are likely to lead to better enzyme replacement therapy as well as rationale design of small molecules for use in chaperone therapy.

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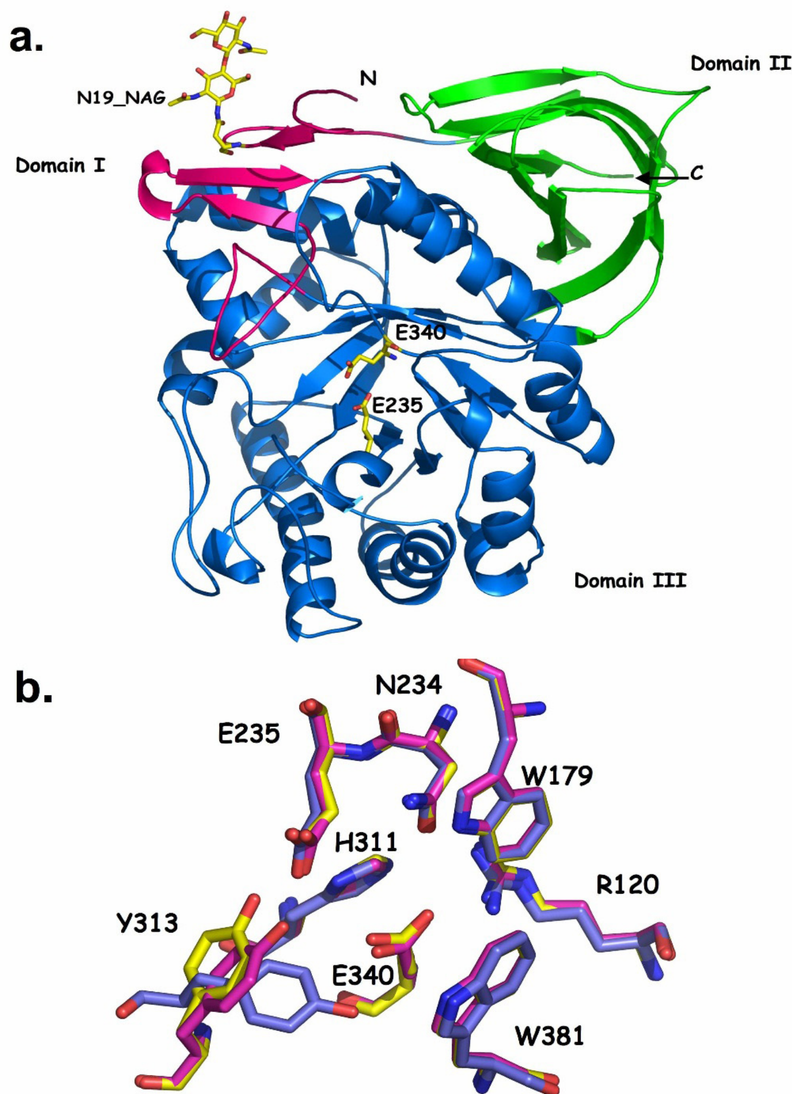

Fig. 3 Three dimensional structure of GlcCerase produced by a plant-cell system (prGCD). (a) Domain I is in pink, domain II in green, and domain III in cyan. The active site residues E235 and E340, are shown as sticks. Glycosylation detected at N19 is also shown. (b) Comparison of the catalytic residues of prGCD (blue), Cerezyme® (pink), and a Cerezyme®/CBE conjugate (yellow). From Shaaltiel et al, 2007

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Acknowledgements

A.H. Futerman is The Joseph Meyerhoff Professor of Biochemistry. Supported by the Israel Science Foundation, the US-Israel Binational Science Foundation, the German-Israel Science Foundation, the Minerva Foundation, the National Institutes of Health, the Children's Gaucher Research Fund, and the Magneton Program, Office of the Chief Scientist, Ministry of Industry and Commerce.

INTERNAL support

Supported by the estate of Louis Uger, Canada, by a research grant from the Irvin and Ruth Swartzberg Family Foundation, and by the Yeda CEO fund.