Sphingolipids in health and disease

Tony Futerman

Yael Pewzner-Jung

Irene Pankova-Kholmyansky

Elena Grimberg

Adi Mesika

Luba Ginzburg

Yaacov Kacher

Sujoy Lahiri

Boris Brumshtein

Sharon Epstein

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 Longevity assurance (LASS) proteins. The first member of this family to be discovered was *LAG1*, shown to be required for ceramide synthesis in yeast. Gene database analyses subsequently revealed a new family of genes containing the Lag1 motif in mammals. Ceramide consists of a sphingoid long chain base to which a fatty acid is attached (Fig. 1); previously it was assumed that there would be one or two ceramide synthase isoforms, but our studies have demonstrated that at least 5 of the 6 known LASS proteins are highly selective towards a defined fatty acid substrate, implying an important role in cell physiology for ceramides containing distinct fatty acids. We have also shown, by biochemical studies, that LASS5 is a *bona fide* ceramide synthase. We are currently determining which regions of the LASS proteins are involved in their activity and specificity, and are also studying the roles of each family member in ceramide-dependent signaling events.

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

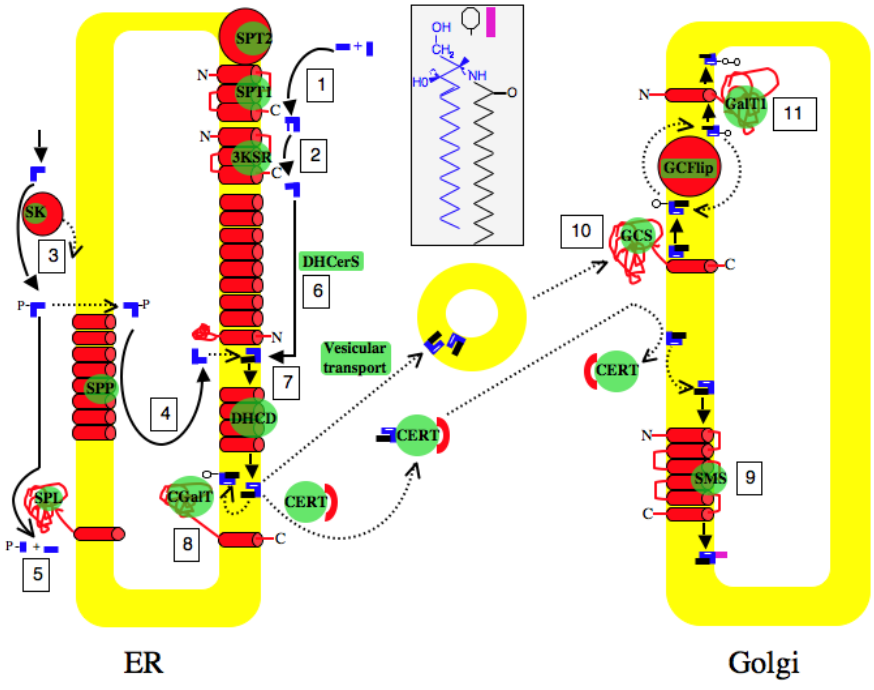

Fig. 1 *The sites and topology of SL metabolism in the ER and Golgi. Solid arrows indicate enzymatic reactions, and dashed arrows indicate transport steps. The topologies of the reactions, where known, are shown in green circles, and steps for which the topology is not known, or the protein not unambiguously identified, as green rectangles. The insert shows the structure of ceramide, and is color-coded according to the SLs shown in the scheme. For more details, see Trends Cell Biol. 15, 312-318.*

Department of Biological Chemistry

972 8 934 2704

972 8 934 4112

tony.futerman@weizmann.ac.il

www.weizmann.ac.il/Biological_Chemistry/scientist/futerman

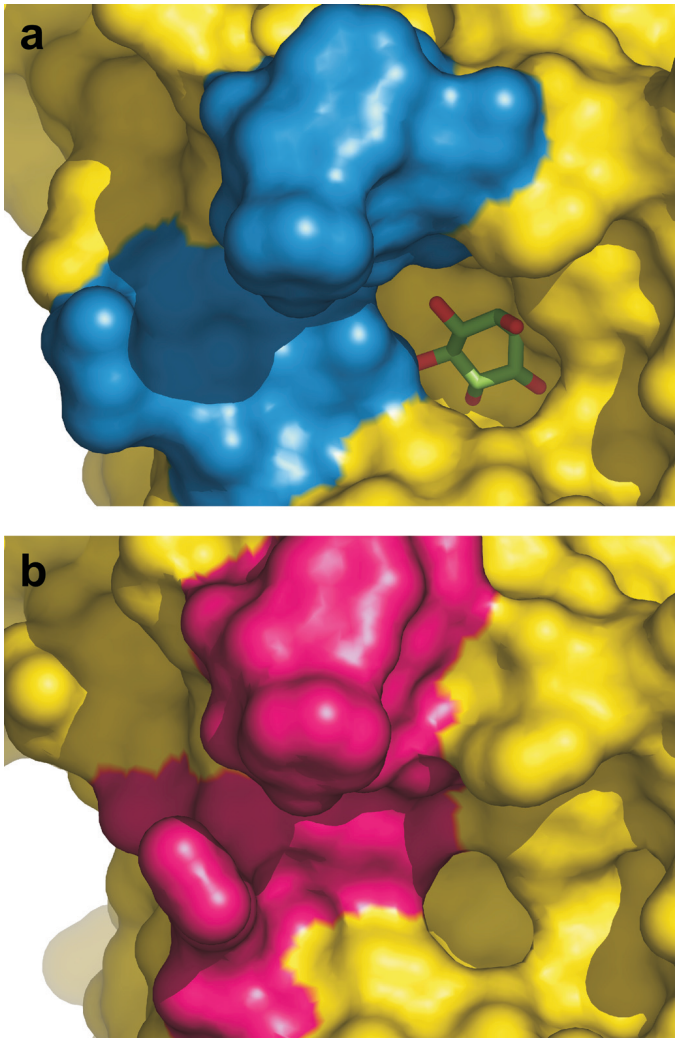

Fig. 2 *Surface of GlcCerase illustrating the open and closed conformations. (a) The open conformation in which CBE is bound to the active site. (b) The closed conformation in which the surface lid restricts access to the active site.*

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. Microsomes prepared from human Gaucher type 2 and 3 brain samples show a similar elevation in Ca^{2+}-release. Cytosolic Ca^{2+}-levels are 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. 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 determined the X-ray structure of acid beta-glucosidase (GlcCerase; Cerezyme®), the enzyme used in enzyme replacement therapy in Gaucher disease. Furthermore, we recently resolved the structure of Cerezyme® conjugated with an irreversible inhibitor, conduritol-B-epoxide (CBE), and were able to determine why mutations in some amino acid residues cause Gaucher disease. Despite the observed overall similarity between the Cerezyme®-CBE structure and that of native Cerezyme®, we detected a significant structural change in two surface loops at the entrance to the active site that act as a lid controlling access to the active site. The discovery of this lid provided the first mechanistic insight into how GlcCerase mutations result in reduced catalytic activity and as a consequence cause Gaucher disease. (Fig. 2). The availability of these structures may now provide the possibility of engineering improved GlcCerase for better enzyme replacement therapy, and of designing structure-based drugs aimed at restoring the activity of defective GlcCerase in Gaucher disease.

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