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Sphingolipids as regulators of neuronal growth and development

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Biological membranes contain three major lipid classes, glycerolipids, sphingolipids (SLs), and sterols. Until recently, most research on SLs focused on their structural roles, or on the consequences of SL accumulation in various inherited metabolic disorders (the sphingolipid storage diseases). However, SL research has undergone a renaissance in the past decade due to the realization that SLs are involved in a number of important regulatory processes. Of these, two have received greatest attention, namely the role of SLs as regulators of membrane traffic, and the their roles as second messengers. A challenge for the new decade in the field of SL research is to clarify the relationship between the various roles proposed for SLs in regulating cell function.

In the past 3-4 years, our laboratory has focused on two major issues. The first concerns the regulation of SL metabolism during cell development, with a view to understanding the function of specific SLs at distinct stages of development. The second concerns the roles that SLs play in regulating neuronal development.

SL metabolism during development

The sites of SL synthesis have been determined by our laboratory and others. SL synthesis begins in the endoplasmic reticulum (ER), but is completed in the Golgi apparatus, where glucosylceramide (GlcCer) and sphingomyelin (SM) are formed from ceramide. Little is known about the regulation of this pathway at the molecular or cellular level. We have shown that the levels and types of SLs change during the development of various cell types and in response to various physiological stresses, due to up- or down-regulation of the activity of various enzymes in the biosynthetic pathway. As one example of many, upon substrate depletion, 3T3 cells up-regulate the activity (by post-transcriptional mechanisms) of three enzyme leading to the synthesis of a relatively minor glycosphingolipid (globoside, Gb3), presumably so as to maintain levels of constant levels of globoside on the cell surface.

The roles of SLs in regulating neuronal development

One of the major interests of our laboratory concerns the relationship between the rate of supply of membrane components and the rate of cell growth, particularly neuronal growth. For these studies, we routinely culture embryonic hippocampal neurons from rats or mice, in such a way that axons and dendrites can be distinguished both morphologically and biochemically. Using these neurons, we have shown that rapid axon growth depends on ongoing GlcCer synthesis. Thus, factors that stimulate axon growth are ineffective when GlcCer synthesis is inhibited, and as might be predicted, the rate of GlcCer synthesis increases when axonal growth is stimulated. Likewise, the rate of dendrite growth can be significantly decreased when SL synthesis is inhibited.

In addition to the need to constantly supply SLs so as to maintain neuronal growth, SL turnover also regulates neuronal development. Originally we showed that ceramide, a key metabolic intermediate in the biosynthetic and degradative pathways of SL metabolism, and recently proposed to be a second messenger, can stimulate the initial stages of neuronal growth. Together with the group of Mike Fainzilber, we have now shown that binding of nerve growth factor to the p75 neurotrophin receptor can stimulate the formation of ceramide from SM, and as a result, accelerate the initial stages of neuronal growth (for more details, see the abstract from Mike Fainzilber's laboratory). Thus, SLs can regulate neuronal growth by either acting as rate limiting components in the supply of new membrane material, or by acting a second messengers when generated at the plasma membrane.

One of the most exciting areas to develop in the laboratory over the past 2-3 years concerns the effects of accumulation of SLs (particularly GlcCer) on neuronal function. In collaboration with Menahem Segal (Dept. of Neurobiology), we demonstrated that GlcCer accumulation in hippocampal neurons causes an increase in ER density and in functional calcium stores. Moreover, the increased levels of release of calcium from the ER results in increased sensitivity of these neurons to a variety of agents that cause neuronal cell death. These data may be of relevance for understanding the pathophysiology of the human inherited metabolic disorder, Gaucher disease, in which cells accumulate GlcCer due to defective activity of the lysosomal hydrolase responsible for degrading GlcCer, glucocerebrosidase. Neuronal dysfunction is observed in some forms of this disease,

but the mechanism by which GlcCer accumulation leads to neuronal dysfunction is completely unknown. This is currently an issue that is being actively studied in the lab. We are extending these studies to other inherited disorders of SL metabolism, including Tay Sachs disease, and for this purpose, are establishing colonies from mouse models of both Gaucher and Tay Sachs disease.

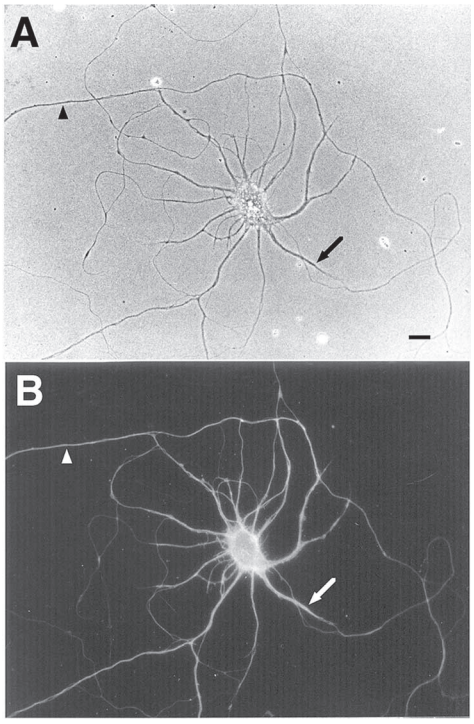

Fig.1. *An example of cultured hippocampal neurons. The top panel shows a phase contrast micrograph of neurons in which axons (arrowhead) and dendrites (arrows) can be distinguished. The bottom panel shows that the glyco-SL, ganglioside GM1, is distributed uniformly over the axon and dendrite surface as judged by binding of a fluorescent analog of cholera toxin (see Shogomori at al.).*

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Acknowledgments

Work in the laboratory is supported by the ISF, BSF, GIF, Heinemann, Mizutani and Taub Foundations.