Short Review
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Glycosphingolipids and Protein Sorting: Rafts and More

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The cell membranes of higher eukaryotes contain a class of lipids that is different from the regular membrane phospholipids in the remarkable variation in its head group structures, the glycosphingolipids. These lipids are anchored to the membrane by a two-tail lipid anchor, ceramide, to which is attached a mono-, di-, or polysaccharide. Most glycosphingolipids contain lactosylceramide, Gal(ß1-4)Glc(ß1-1)ceramide, to which a number of other carbohydrates can be attached by various different glycosidic linkages. The enormous range of headgroup structures and their occurrence on the cell surface already suggested that they function in specific recognition between cells and subsequent signaling towards the cell interior[1]. However, the molecular mechanism of signaling only became clear when it was realized that the ceramide lipid backbone provides these lipids with special physical properties. In the presence of cholesterol, they can segregate from the bulk of the membrane phospholipids that is based on a diacylglycerol backbone into a more ordered, but still fluid, domain. With the glycosphingolipids, the ceramide-based phospholipid sphingomyelin also partitions into these domains, which may cover a large fraction of the cell surface. By mechanisms that are largely unknown, membrane proteins involved in signaling collect in the domains (or "rafts") and, because this partitioning depends on the signaling state of the protein, rafts are essential in bringing signaling proteins together (e.g., [2]). One of the greatest unknowns concerning these rafts is the lipid and protein organization on the cytosolic surface[3].

In a recent paper[4], the groups of Peter van der Sluijs and myself demonstrated that in pigment cells, glycosphingolipids are required for the transport of newly synthesized enzymes from the Golgi complex to the melanosomes, where they are required for the synthesis of the melanin pigment. Work on the intracellular sorting of glycosphingolipids had initially suggested

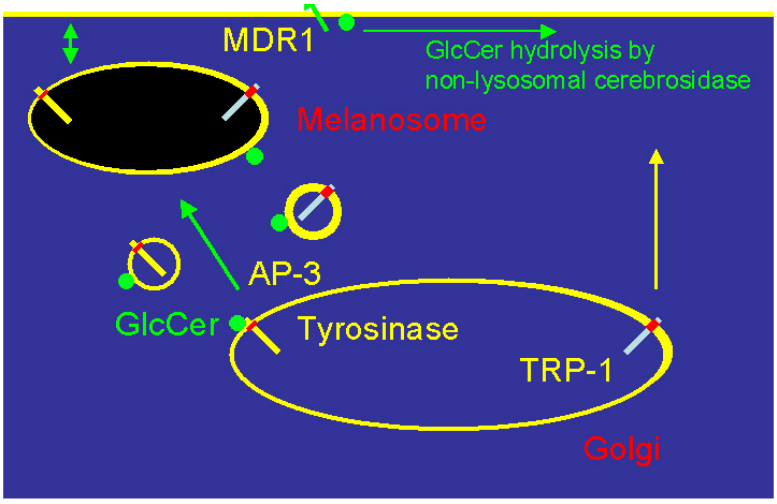

FIGURE 1. In the trans Golgi network of melanocytes, tyrosinase and TRP-1 are incorporated into budding vesicles destined for the melanosome, a pathway that depends on the coat adaptor protein AP-3. Glucosylceramide (GlcCer) is required for this budding step[4]. Since it is synthesized on the cytosolic aspect of the Golgi, it may be directly involved in coat assembly. After serving its purpose, it may be removed from the cytosolic surface by either the translocator MDR1[9] or by a glucoceramidase[11]. TRP1 has a longer hydrophobic transmembrane domain and may follow a second glucosylceramide-dependent pathway to the melanosome. Only in the absence of glucosylceramide it is transported to the plasma membrane (yellow arrow)[4].

that the sorting of membrane proteins to the apical membrane of epithelial cells was mediated by their partitioning into glycolipid microdomains on the lumenal aspect of the Golgi membrane[5]. However, the present data indicate that the glycosphingolipid-dependent sorting mechanism in pigment cells does not involve lumenal glycosphingolipid rafts. In contrast, they suggest the possibility that the simple glycosphingolipid glucosylceramide functions on the cytosolic surface of, most likely, the Golgi complex in the budding of the transport vesicles destined for the melanosomes.

All complex glycosphingolipids are assembled by the stepwise addition of sugars to glucosylceramide in the lumen of the Golgi. However, biochemical studies on the synthesis of glucosylceramide and, later, the cloning of the glucosyltransferase demonstrated that this glycolipid is synthesized on the Golgi's cytosolic surface[6]. Thus, glucosylceramide must translocate across the Golgi membrane to gain access to the lactosylceramide synthase. Generally, it has been observed that this is mediated by an energy-independent translocase. However, we have found that glucosylceramide (analogs) can be translocated across the plasma membrane by one or more ATP-binding cassette (ABC) transporters[7], which suggests that this lipid may serve a function on the cytosolic surface of the organelles between the sites of synthesis (Golgi) and translocation (plasma membrane).

Mice with null-alleles for the glucosyltransferase were not viable[8], but a cell mutant unable to synthesize glucosylceramide by lack of glucosyltransferase activity is[6]. A clear phenotype in these cells was found much later, when Hein Sprong noted that the mutant cells were white while the parental melanoma cells were black. He showed that all enzymes required for pigmentation were present in the mutant cells, but that the first and rate-limiting enzyme in the pathway, tyrosinase, was mislocalized. Instead of being in the melanosomes it was located in the Golgi. A second melanosomal enzyme, TRP1, which escaped the Golgi of the cell mutant, traveled to the plasma membrane. When, from a sequence comparison, a tyrosinase was constructed that escaped the Golgi, the mutant cells made pigment. This demonstrated that glycosphingolipids were exclusively needed for transporting tyrosinase out of the Golgi. Transfection of the cells with the glucosyltransferase restored tyrosinase transport. However, the exogenous addition of the enzyme's product, glucosylceramide, did not. Transport was restored when glucosylsphingosine, which lacks one fatty tail, was fed to the mutant cells. Glucosylsphingosine was converted by the cells to glucosylceramide, and both lipids, when added

exogenously, reached the lumen of the Golgi where they were converted to lactosylceramide. Apparently, this was irrelevant for restoring pigmentation. Since glucosylsphingosine is converted to glucosylceramide on the cytosolic surface, one possibility is that glucosylceramide on the cytosolic surface is the relevant parameter for restoring transport. However, it cannot be excluded that glucosylsphingosine by itself is the active species.

A glycosphingolipid is required for the vesicular pathway from the Golgi to the melanosome. Since this pathway involves the budding of carrier vesicles from the trans Golgi, the simplest mechanism would be that the lipid is required for the formation of a functional protein coat on the cytosolic surface of the Golgi. After budding and transport to the melanosome the lipid (most likely glucosylceramide) might be removed from the cytosolic surface by a translocator[9], which would make the process unidirectional. A defect in this machinery may lie at the basis of some forms of the Hermansky-Pudlak syndrome, a group of related recessively inherited human pigmentation disorders[10]. Finally, melanosomes are part of the endosomal membrane system. It will be interesting to see the consequences of the absence of glycosphingolipids on endosome structure and function in regular cells that do not make pigment.

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