Ceramide trafficking: learning from the rebels

For a relatively long time now, many advances in cell biology used to take place like this : someone performed a screen in yeast or flies, and found a set of genes or complementation groups; then, someone else developed an assay in mammalian cells for the same phenomenon, and identified candidate genes, and made a connection. Then the connections remained speculative until someone else came along and sequenced large sets of genes or genomes, illuminating entire pathways that were conserved, or not. It was a good arrangement, and everyone was happy – biology was moving at a frightening rate already.

However, all along, there have been rebels who have believed that: (i) mammalian cells do a lot of things differently from yeast and flies, and not all of these things can be understood from a screen done in another system; (ii) one can do a screen in mammalian cells, even if the technology of the time is limiting. As it stood then, their basic philosophy was probably not wrong, but without the runaway results that one could get from a classical screen, nobody joined the gang. Things though, have changed now – technology is no longer limiting – and the rebels are starting to come forth.

In a proof of concept article, Hanada and colleagues (Hanada *et al* 2003; Munro 2003; Riezman and van Meer 2004) designed a mammalian cell screen to study how the levels of sphingolipids are regulated. Sphingolipids are synthesized from a precursor molecule called ceramide in the Golgi complex and are common constituents of plasma membranes. The authors exploited the properties of a toxin derived from earthworms called lysenin. Lysenin binds very specifically to sphingomyelin, a sphingolipid that is a major component of the outer bilayer of the plasma membrane, and kills cells by perforation. By screening mutagenized mammalian cells for resistance to the toxin lysenin, the authors expected to find mutants that had defects in the trafficking of sphingomyelin to the cell surface. They succeeded, and were able to complement one such resistant mutant using highly efficient virus-mediated gene delivery and expression. They showed that the mutation conferring lysenin resistance was in a gene coding for a ceramide transfer protein (CERT). CERT functions at endoplasmic reticulum (ER) membranes by extracting ceramide and transferring it to the Golgi complex for conversion into sphingolipids (see figure 1). The mutant CERT could not localize to the ER correctly, thus less

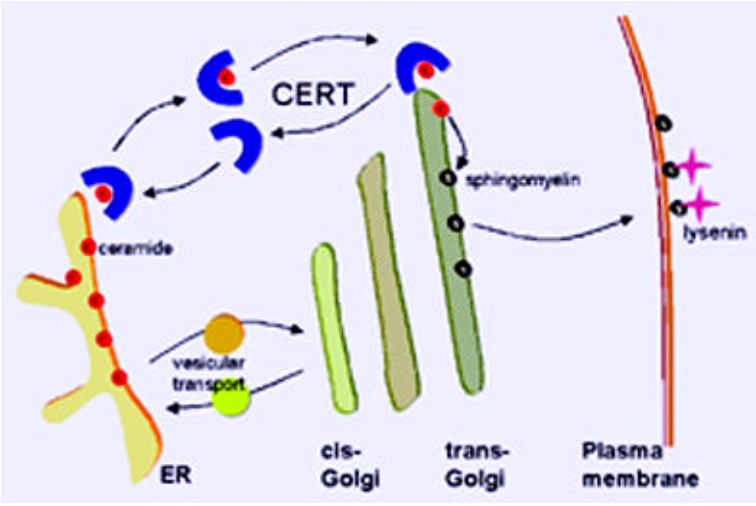

Figure 1. CERT transfers ceramide from the ER to the Golgi complex, where it is converted to sphingomyelin, and trafficked to the plasma membrane.

ceramide was available for conversion to sphingomyelin at the Golgi complex. Hence, these mutants had low levels of sphingomyelin on their surface and were resistant to lysenin.

While the technique of using a mammalian cell culture screen to isolate novel genes was both elegant and unique, the discovery of CERT in itself has profound implications for cellular traffic. Typically, protein and lipid cargo is carried to the Golgi complex from the ER via transport vesicles. However, when ER-Golgi vesicular transport is blocked, ceramide continues to reach the Golgi complex (Kok *et al* 1998). This non-vesicular pathway for transport of ceramide is poorly understood, and CERT is the first molecule found that is likely to function in this regard (see figure 1). There are other proteins in mammalian cells with lipid- and organelle-binding domains that are similar to those in CERT. Therefore CERT may define a family of proteins that transfer lipids between organelles without relying on vesicles.

All these details of lipid trafficking are of no small consequence for keeping the cell alive and disease-free. Both ceramide and sphingolipids are signalling molecules that partake in major cellular pathways controlling growth, response to stress and death. The rebel screen has provided the basis for unraveling an entirely new aspect of cellular lipid homeostasis.

References

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