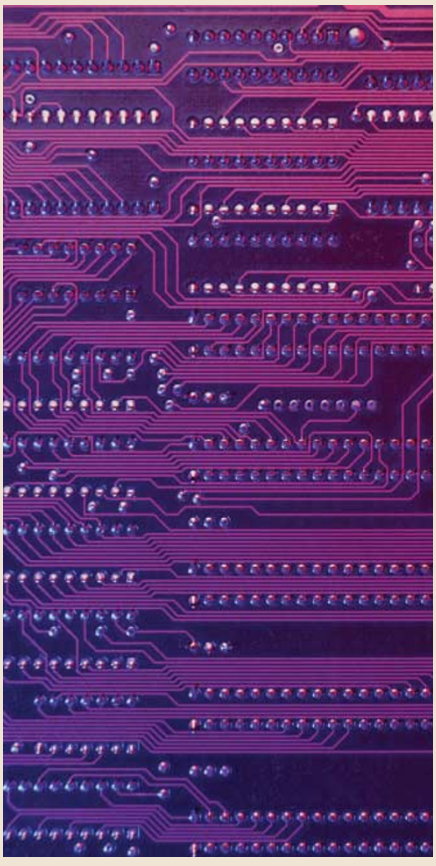
encoding a protein that strongly resembles a MAPKK kinase. The *yda* mutations are expected to negatively affect kinase activity. The authors also created deletions on the amino-terminal side of the catalytic domain of YDA, as this region is often involved in repressing catalytic activity. These alleles complemented the mutant phenotype, but also gave rise to new gain-of-function phenotypes. Embryos that had amino-terminal deletions of *YDA* generally developed slightly larger basal cells and larger suspensors. In the most extreme cases, development of the pro-embryo was completely inhibited.

So the authors propose that YDA acts as part of a cell-fate switch that promotes extra-embryonic fate. The next challenge is to identify the upstream and downstream components of this signalling pathway.

Katrin Bussell

References and links

ORIGINAL RESEARCH PAPER Lukowitz, W. *et al.* A MAPKK kinase gene regulates extra-embryonic cell fate in *Arabidopsis*. *Cell* **116**, 109–119 (2004)



LIPID TRAFFICKING

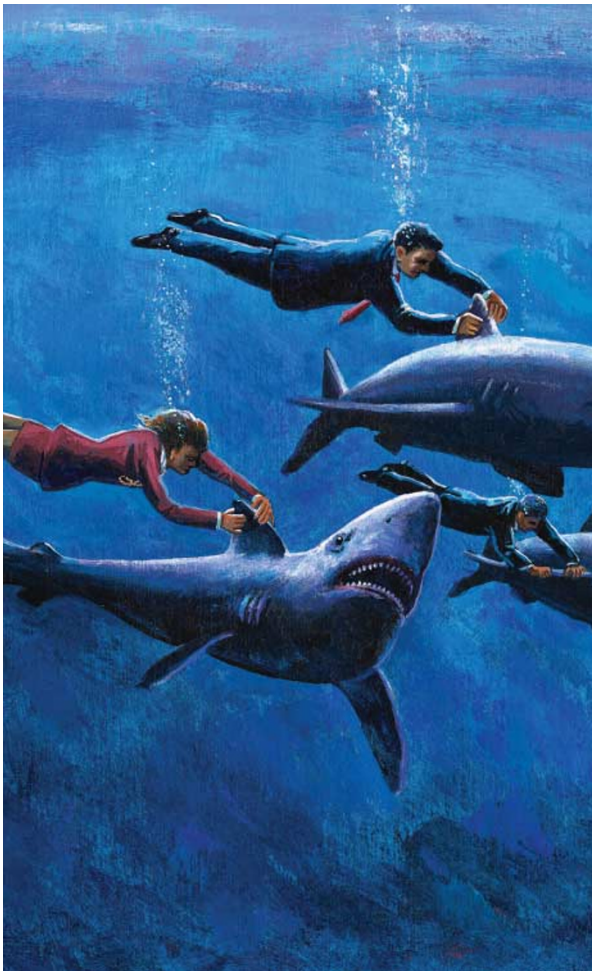
A new way to travel

The biosynthesis of lipids involves steps that occur in different intracellular compartments, and the intracellular movement of lipids might also be important in lipid-mediated signalling. However, the intracellular trafficking of lipids remains poorly understood, although it has been suggested to occur by both vesicle-dependent and -independent mechanisms. Now, though, in *Nature*, Hanada *et al.* provide new insights by describing a protein that mediates the ATP-dependent transport of ceramide from the endoplasmic reticulum (ER) to the Golgi in a vesicle-independent manner.

Ceramide is made in the ER and is then transported to the Golgi, where it is converted to sphingomyelin. In the Chinese-hamster-ovary mutant cell line LY-A, the main pathway of ceramide transport to the Golgi is genetically impaired, with no defect in the ER-to-Golgi trafficking of proteins. The authors therefore began by using functional-rescue experiments to show that a protein, which they named CERT, is defective in this cell line. Human CERT is identical to a splice variant of human Goodpasture antigen-binding protein (GPBPΔ26).

LY-A cells are defective in converting ceramide to sphingomyelin, and Hanada *et al.* showed that this defect could be rescued by transfecting these cells with human CERT or the larger splice variant GBPB/CERT$_L$. In addition, using a fluorescent analogue of ceramide, they showed that, as expected, the ER-to-Golgi trafficking of ceramide in LY-A cells was impaired, but that it became nearly identical to that in wild-type cells when LY-A cells were transfected with human CERT. Furthermore, they showed that depleting intracellular ATP levels impaired the ER-to-Golgi trafficking of ceramide in wild-type cells, LY-A cells and LY-A cells transfected with human CERT. It therefore seems that CERT and CERT$_L$ are involved in the ATP-dependent ER-to-Golgi transport of ceramide in LY-A cells.

CERT contains a phosphoinositide-binding pleckstrin-homology (PH) domain (which targets CERT to the Golgi by binding phosphatidylinositol-4-phosphate (PtdIns4P)), a middle region, and a putative lipid-transfer-catalysing domain called START. The authors showed that CERT and CERT$_L$ can specifically extract ceramide from phospholipid bilayers in a START-domain-dependent manner in a cell-free system, and that CERT interacts with ER membranes and specifically extracts ceramide. Furthermore, they showed that, *in vitro*, CERT can catalyse both the specific extraction of ceramide from donor vesicles and its transfer to acceptor vesicles in a START-domain-dependent manner.



Hanada *et al.* next showed that mutation of a conserved glycine in the PH domain of CERT is responsible for the phenotype of LY-A cells, and that this mutation disrupts PtdIns4P recognition. When they compared the intracellular distribution of CERT and mutant CERT by fusing them to green fluorescent protein, they found that the mutation impaired Golgi association. It therefore seems that CERT can associate with the Golgi in a manner that depends on the recognition of PtdIns4P by its PH domain.

This work has therefore showed that CERT mediates the ATP-dependent ER-to-Golgi transfer of ceramide in a non-vesicular manner. The START domain of CERT specifically recognizes CERT and catalyses its intermembrane transfer, whereas the PH domain can target CERT to the Golgi. In addition, the middle region of CERT contains an ER-targeting motif. To the authors' knowledge, this work "…is the first example of the molecular identification of a specific lipid-sorting factor that operates in the synthesis of membrane phospholipids".

Rachel Smallridge

References and links

ORIGINAL RESEARCH PAPER Hanada, K. *et al.* Molecular machinery for non-vesicular trafficking of ceramide. *Nature* **426**, 803–809 (2003)