From the Archive

Lipid raft idea is floated

The introduction of a fluorescent lipid probe, specifically fluorescent ceramide, in the mid-1980s (Lipsky and Pagano, 1983; Lipsky and Pagano, 1985) gave Gerrit van Meer and Kai Simons just the tool they needed to attack a nagging question.

Lipid locations

For a decade, it had been known that the apical and basolateral membranes of epithelial cells had different lipid compositions (Kawai, 1974), and specifically that glycolipids are enriched apically. In 1981, the tight junction was proposed as the barrier that kept these two membrane populations distinct (Dragsten et al., 1981). Playing off a finding that different viruses budded

Lipids pause in the Golgi before being sorted to distinct destinations.

VAN MEER

from the different poles of cultured epithelial cells (Boulan and Sabatini, 1978), van Meer and Simons showed in 1982 that the envelopes of those viruses contained different lipid compositions (van Meer and Simons, 1982).

At about the same time, others had shown that heterogeneous lipid domains existed (Karnovsky et al., 1982) and that glycosphingolipids clustered (Thompson and Tillack, 1985) in both model membrane systems and biological membranes. This suggested that lipid domains might affect membrane functions and structure, but real evidence of a biological role was lacking.

Because plasma membrane lipids are synthesized intracellularly, van Meer and Simons reasoned that lipid sorting must take place to set up the epithelial cell membrane domains. The NBD–ceramide probe offered a handy way to start on the project because, once it was inside cells, it would be converted into two distinct lipid probes: an NBD–sphingomyelin and an NBD–glucosylceramide, analogues of a basolateral and apical lipid, respectively. The conversion

occurred in the Golgi and then the fluorescent probes could be followed to plasma membrane destinations.

While he was a post-doc with Simons at the EMBL in Heidelberg, Germany, van Meer quantified the sorting using the NBD-labeled probes. Using Madin-Darby canine kidney (MDCK) epithelial cells grown on filters, he used "back exchange" with serum albumin applied to either side of the filter to extract and measure the fluorescent lipids that sorted to either the apical or basolateral side (van Meer et al., 1987). He found that the NBD–glucosylceramide was enriched two to four times on the apical membrane, whereas the NBD–sphingomyelin was equally distributed between the apical and basolateral sides. This process quantitatively accounted for the in vivo lipid distribution.

If transport, then rafts

"This was the first piece of evidence that we were on the right track," says Simons. He notes that, until this point, lipid microdomains had been reported as biophysical phenomena, but the cell had not previously been caught in the process of actively setting up these differences. From this paper, a model emerged that would be the first tip of a lipid microdomain, or raft, iceberg. The paper provided data that lipids are potentially sorted in the Golgi complex. Based on the physical properties of glycolipids, which suggested they could associate with each other, "the apical sorting platform idea took form," says Simons.

The lipids wound up on the exoplasmic leaflet of the plasma membrane where they could not diffuse past tight junctions, so it seemed logical that they might be synthesized on the lumenal leaflet of the Golgi and transported to the plasma membrane via vesicles. It was at this point that the authors touched ever-so-briefly on the topic of lipid subdomains. Lipid "sorting," they stated, "must involve the lateral segregation in this leaflet of lipids into those areas of the membrane that will bud to form transport vesicles destined for either the apical or basolateral plasma membrane domain. The factors involved in this segregation process are unknown."

The idea that lipid microdomains might exist within a continuous "fluid" membrane was radical then and continues to be controversial now. The following year, Michael Lisanti, Enrique Rodriguez-Boulan, and colleagues showed that six glycosyl-phosphatidylinositol (GPI)–anchored proteins followed the same apical distribution pattern as the NBD–glycolipid (Lisanti et al., 1988). But it wasn't until 1992, van Meer says, that a "really crucial paper sent the thing off."

Rafts in vitro

Deborah Brown and Jack Rose discovered that cold detergent extraction allowed the isolation of membranes enriched in glycosphingolipids and GPI-anchored proteins (Brown and Rose, 1992). These detergent-resistant membranes (DRMs) literally floated like rafts to the top of the preparations, and the simple

technique "opened the field up to experimental analysis," says van Meer. A few years later, Brown and colleagues proposed that the structure of the DRMs was due to a separate, less fluid membrane phase regulated by tight packing of fatty acyl chains and cholesterol (Schroeder et al., 1994).

Many later studies on DRMs showed that raft-associated proteins were important for cell signaling and that movement of those proteins into the DRM fraction was associated with signal activation (for review see Anderson and Jacobson, 2002). But the harsh in vitro techniques of isolating DRMs and depleting membranes of cholesterol have brought much criticism to the field about artifactual or at least, nonphysiological, results (Lai, 2003). Pinning down what rafts look like and how they act in real time in vivo has been slippery. Most researchers agree that, if rafts exist, they are extremely small (25–100 nm) and transient. The technology for observing lipids in unperturbed, living cells has yet to catch up.

Ken Jacobson says "the raft concept looks good from our work on model membranes" showing that raft domains depend on cholesterol density and that GPI-anchored proteins partition into rafts (Dietrich et al., 2001). "But what is, if any, the in vivo correlate?," he asks. "The membrane is a liquid structure and we are still learning how to derive structural information. There has to be lateral heterogeneity, but figuring out how to really prove that in a compelling way still remains the challenge." Brown suggests that raft formation might be regulated so that "the membrane composition is poised at the brink of raft formation and you need to flip a switch." These stabilized rafts almost certainly function in membrane trafficking, virus budding, and signal transduction. **KP**

Anderson, R. G., and K. Jacobson. 2002. *Science*. 296:1821–1825.

Boulan, E.R., and D.D. Sabatini. 1978. *Proc. Natl. Acad. Sci.* 75:5071–5075.

Brown, D.A., and J.K. Rose. 1992. *Cell*. 68:533–544.

Dietrich, C. et al. 2001. *Proc. Natl. Acad. Sci.* 98:10517–10518.

Dragsten, P.R. et al. 1981. *Nature*. 294:718–722.

Karnovsky, M.J. et al. 1982. *J. Cell Biol*. 94:1–6.

Kawai, K. et al. 1974. *Biochim. Biophys. Acta*. 369:222–233.

Lai, E.C. 2003. *J. Cell Biol*. 162:365–370.

Lipsky, N.G., and R.E. Pagano. 1983. *Proc. Natl. Acad. Sci.* 80:2608–2612.

Lipsky, N.G., and R.E. Pagano. 1985. *J. Cell Biol*. 100:27–34.

Lisanti, M.P. et al. 1988. *Proc. Natl. Acad. Sci.* 85:9557–9561.

Schroeder, R. et al. 1994. *Proc. Natl. Acad. Sci.* 91:12130–12134.

Thompson, T.E., and T.W. Tillack. 1985. *Annu. Rev. Biophys. Biophys. Chem.* 14:361–386.

van Meer, G., and K. Simons. 1982. *EMBO J.* 1:847–852.

van Meer, G. et al. 1987. *J. Cell Biol*. 105:1623–1635.

Making tendons

Cells are fastidious about their internal conditions. But for scientists trying to decipher how collagen forms structures such as tendons and the cornea, the big question 30 years ago was how much control cells exert over their surroundings. In a tendon, for example, collagen molecules join end-to-end to yield fibrils, which line up alongside one another to create bundles. These amalgamations, in turn, cluster into fascicles. Most researchers thought that cells did little to aid the process beyond manufacturing collagen, according to Robert Trelstad (Robert Wood Johnson Medical School, Piscataway, NJ). The prevailing view, he says, "was that all the cell had to do was squirt this stuff [collagen molecules] into the intercellular space and—voila!—it would self-assemble." Trelstad expressed his disagreement with that explanation in a ditty:

Self-assembly is a thought
That's inherently complete
You merely have to state it once
It'll automatically repeat, repeat, repeat…
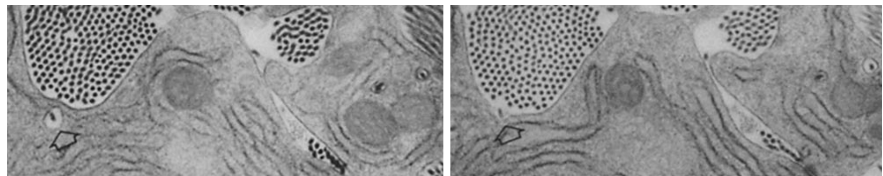
An individual collagen fibril (open arrow, left) joins a bundle (right).

It took a decade to amass evidence that cells take a more active role. In the mid-1970s, his group's in vitro study revealed that collagen doesn't condense into fibrils in one fell swoop, as many researchers had argued. Instead, fibrils form in stages (Trelstad et al., 1976). Ten years later, he and colleague David Birk trained one of the new high-voltage electron microscopes on embryonic chick tendons. They identified three kinds of pockets in the membranes of collagen-producing fibroblasts (Birk and Trelstad, 1986). One was a deep, narrow crevice that held a single fibril, like a hair in a follicle. The second, wider groove cradled fibril bundles, while the third, even larger indentation held clusters of bundles. The observations suggested that the cell was orchestrating collagen condensation and fibril grouping by adjusting the contours of its membrane. Trelstad and Birk hypothesized that the vesicles that carry newly synthesized collagen stack up and then merge to form the deep crevices, where collagen molecules interlink into a fibril. The cell then manipulates the membrane that separates the pockets, allowing fibrils to merge into bundles and bundles to band together. When it comes to making a tendon, "cellular control of the early stages is essential," Trelstad says. A recent study expanded on the findings, showing how the cell guides the fibrils into place using long protrusions termed fibropositors (Canty et al., 2004). **ML**

Birk, D.E., and R. L Trelstad. 1986. *J. Cell Biol*. 103: 231–240.

Canty, E.G., et al. 2004. *J. Cell Biol*. 165: 553–563.

Trelstad, R. L., K. Hayashi, and J. Gross. 1976. *Proc. Natl. Acad. Sci. U.S.A.* 73: 4027–4031.