IMAGING AND VISUALIZATION

SIMS for membranes

The combination of two imaging methods, each with its own limitations, brings researchers closer to the goal of describing the complexity of membrane organization with high spatial resolution and a high degree of chemical information.

The simplest way to describe a membrane is as a lipid bilayer, but it is actually a complex network of lipid, protein and sugar components, the organization of which is thought to have functional significance for the cell. Although it is possible to 'see' small subsets of these components—such as a complex of membrane receptors—by X-ray crystallography, methods that allow greater lateral resolution are necessary to study entire membrane domains. Fluorescence microscopy is often used to image differentially labeled components at such a resolution, but addition of fluorescent labels can change the physical properties of the relatively small components of biological membranes.

Thus, to measure model membrane composition, Steven Boxer of Stanford University and his colleagues turned to secondary ion mass spectrometry (SIMS), a method originally developed over 50 years ago. They used a high-resolution version of this technology, NanoSIMS, to analyze supported bilayers of two lipids labeled with different stable isotopes (Kraft *et al.*, 2006). In this technique, the sample is bombarded with a beam of ions, and the secondary ions emitted from the sample are extracted and measured by mass spectrometry (**Fig. 1**). The resulting secondary ion data for each of the isotopes can be used to create an image of the distribution of the two lipids in the bilayer.

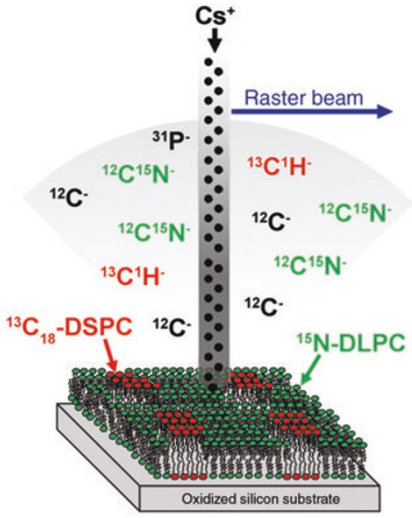

Figure 1 | Schematic of the NanoSIMS analysis. As the raster beam passes across the supported lipid bilayer containing domains of two labeled lipids, $^{13}C_{18}$-DSPC and ^{15}N-DLPC, secondary ions are generated. Ions characteristic of each lipid (in red and green, respectively) can be used to identify these lipids in the NanoSIMS image to create a chemical composition map of the surface. Reprinted with permission from AAAS.

In analyzing the NanoSIMS data, Boxer's team found that the lipid distribution varied significantly in some of the domains. The NanoSIMS data also helped explain some of the irregularities observed in the atomic force microscopy (AFM) analysis of the same samples. AFM, which provides information about the 'bumps and lumps' in samples, indicated that there were some structures of unknown origin within the observed domains. Comparison to NanoSIMS data indicated that the observed objects were not subdomains because they did not have the chemical signatures of either of the labeled lipids, and the authors were able to conclude that

STEM CELLS

ADULT STEM CELLS COME UP SHORT

New research demonstrates that terminally differentiated cells are more efficient than adult stem cells for cloning.

Although the cloning of an entire living organism is regarded by many as a Pandora's box, most attempts at cloning have had low rates of success because the rules regarding cloning efficiency remain in large part a black box. Cloning is often performed using a technique called somatic cell nuclear transfer (SCNT), in which donor DNA from one somatic cell is injected into an egg whose DNA has been removed. One important variable to consider when cloning is the source of the donor DNA. DNA taken from undifferentiated embryonic stem cells is regarded as being relatively efficient for cloning, whereas DNA taken from the cells of adult organisms is generally much less so.

Important questions remain, though, as to whether some adult cell types are more effective than others for cloning. A small number of cells in the adult, called adult stem cells, are less differentiated than other types of adult cells, and it is thought these adult stem cells may be better sources of donor DNA for cloning than the more differentiated cell types they give rise to. To directly study this issue, a team of scientists led by Tao Cheng at the University of Pittsburgh School of Medicine and Xiangzhong Yang at the University of Connecticut performed a side-by-side comparison using mouse cells, in which they compared the cloning efficiency of adult stem cells to that of differentiated adult cells.

For their study, Cheng and colleagues used the hematopoietic cell lineage, which is a well-studied lineage that produces blood cells. Hematopoietic stem cells (HSCs) are the best-studied adult stem cells and the most primitive cell type in the hematopoietic lineage; granulocytes, with easily identifiable morphology, are one type of terminally differentiated white blood cells that develop from HSCs. Using a previously established method, which is based on the recognition of unique surface proteins that distinguish HSCs from granulocytes, the authors were able to obtain highly pure populations of both granulocytes and HSCs. When they carried out parallel cloning experiments, they found that cloning with the differentiated granulocytes was actually much more efficient than with the HSCs. This finding was quite unexpected, as Cheng explains, "Given the known very low efficiency of SCNT, it had been hypothesized in the field that the use of purified adult stem cells may improve the efficiency. We thus expected a higher cloning efficiency from HSCs than granulocytes when we began our experiments. So our result is surprising."

they were debris—just dirt.

Taken together, data produced by these two methods provide a clearer picture of the system. "The combination is more powerful than either technique by itself," says Boxer. AFM gives a very high degree of lateral information, "much higher than any version of imaging mass spectrometry can now or probably will ever give, … and it's fantastic for looking at topography, but it does not tell you at all what the molecules are. And mass spectrometry in its many variations, including [NanoSIMS], is the ideal method to analyze what's there because it gives you a chemical map of what's there."

Just as the combination of imaging techniques provides the most information about the membrane, different methods of preparing the membrane for imaging will aid this effort as well. Some groups are now developing methods to image membrane components in a nonsupported format (see, for example, Gonçalves *et al.*, 2006). Boxer and colleagues continue to develop the supported bilayer system with the hope of building up to a more complex system. Other groups are developing ways of removing a part of a membrane from a cell and putting it on a surface. This is one of the avenues Boxer's team is pursuing, and he adds that "if one could do this quickly enough, so that the molecules have not moved very far on the time scale of this ripping-apart process, then the hope is that one can interrogate the lateral composition of membranes derived from real cells. That is the dream experiment, in a sense. It just requires many technical developments."

Irene Kaganman

RESEARCH PAPERS

Gonçalves, R.P. *et al.* Two-chamber AFM: probing membrane proteins separating two aqueous compartments. *Nat. Methods* **3**, 1007–1012 (2006).

Kraft, M.L. *et al.* Phase separation of lipid membranes analyzed with high-resolution secondary ion mass spectrometry. *Science* **313**, 1948–1951 (2006).

To search for clues to help explain this unexpected result, Cheng's team performed transcriptional profiling on the cellular populations. They found that a number of genes, including some involved in epigenetic modification, were differentially expressed between the granulocytes and the HSCs. Although the reason why the HSCs had a lower cloning efficiency than the granulocytes is not completely clear, Cheng believes one possible explanation for this may involve epigenetics: "We speculate that unlike embryonic stem cells, HSCs have a unique epigenetic makeup that prevents their genome from being accessible to many crucial reprogramming factors in the oocytes." Although more experiments are needed to fully explain their findings, the work of Cheng and colleagues could set the stage for easier and more efficient methods to perform cloning experiments, especially when it comes to therapeutic cloning. Cheng explains, "Differentiated cells are much more abundant than adult stem cells in all tissues. So perhaps people do not have to isolate the rare adult stem cells, which is an obviously difficult thing to do, for therapeutic cloning in the future, if the validity of the SCNT-derived [embryonic stem] cells lines from some differentiated cells such as granulocytes can be further demonstrated."

Jesse Potash

RESEARCH PAPERS

Sung, L.Y., *et al.* Differentiated cells are more efficient than adult stem cells for cloning by somatic cell nuclear transfer. *Nat. Genet.*; published online 1 October 2006.

NEWS IN BRIEF

MICROARRAYS

Identifying transcription-factor binding sites

Unraveling the complex circuitry that underlies cellular transcription is a major challenge facing biologists; now a new tool will offer much-needed assistance in tackling this problem. Berger *et al.* have developed a specialized protein binding microarray, comprised of short, carefully selected DNA sequences, that can be used to identity the unique DNA sequences to which a particular transcription factor binds.

Berger, M.F. *et al. Nat. Biotechnol.*; published online 24 September 2006.

PROTEIN BIOCHEMISTRY

Predicting protein orientation on surfaces

Protein immobilization on surfaces is necessary for the use of research tools like protein microarrays. The orientation of a protein on the surface is crucial for its proper functioning, but it is extremely difficult to determine with available experimental approaches. Talasaz *et al.* now describe a computational structural simulation method that can be used to predict protein orientation on surfaces using electrostatic interaction information.

Talasaz, A.H. *et al. Proc. Natl. Acad. Sci. USA* **103**, 14773–14778 (2006).

SPECTROSCOPY

Extending the limits of top-down proteomics

In the 'top-down' approach to mass spectrometry–based protein analysis, intact protein ions are introduced via electrospray ionization into a mass spectrometer, where they undergo fragmentation and subsequent detection. Larger proteins, however, could not be readily analyzed using this method. By applying more energetic conditions during fragmentation, Han *et al.* now show that the top-down approach can be used to fragment proteins as large as 229 kDa. This should help extend the application of top-down approaches to broader proteomic studies.

Han, X. *et al. Science* **314**, 109–112 (2006).

RNA INTERFERENCE

Faster cloning of inverted repeats for RNAi

Cloning of inverted repeats is often the bottleneck in RNA interference experiments. Bao and Cagan constructed pGEM-WIZ, a 3.1-kb vector for assembling inverted repeats for any *Drosophila* sp. gene. The smaller plasmid size allows a clone with an insert to be distinguished from the vector by size, and plasmids containing an inverted repeat, which replicate more slowly in *Escherichia coli*, can be quickly identified by their lower copy number.

Bao, S. & Cagan, R. *RNA* **12**, 2020–2024 (2006).

CHEMICAL TOOLS

Replacement surgery with unnatural amino acids

To substitute a 'key' aromatic residue in the lock-and-key joint of the glutathione transferase enzyme, Hegazy *et al.* turned to combinatorial chemistry. They replaced the key tyrosine with a cysteine and then chemically derivatized this residue to obtain enzyme variants with a wide range of catalytic activities and affinities for the substrate—providing an alternative method for the introduction of unnatural amino acids.

Hegazy, U.M. *et al. Chem. Biol.* 13, 929–936 (2006).