

Three-dimensional imaging of cholesterol and sphingolipids within a Madin-Darby canine kidney cell

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Metabolic stable isotope incorporation and secondary ion mass spectrometry (SIMS) depth profiling performed on a Cameca NanoSIMS 50 were used to image the ¹⁸O-cholesterol and ¹⁵N-sphingolipid distributions within a portion of a Madin-Darby canine kidney (MDCK) cell. Three-dimensional representations of the component-specific isotope distributions show clearly defined regions of ¹⁸O-cholesterol and ¹⁵N-sphingolipid enrichment that seem to be separate subcellular compartments. The low levels of nitrogen-containing secondary ions detected at the ¹⁸O-enriched regions suggest that these ¹⁸O-cholesterol-rich structures may be lipid droplets, which have a core consisting of cholesterol esters and triacylglycerides. © 2016 American Vacuum Society. [<http://dx.doi.org/10.1116/1.4939681>]

I. INTRODUCTION

Lipids and cholesterol comprise cellular membranes and also play important roles in signal transduction^{1,2} and intracellular trafficking.^{3,4} Alterations in the distributions of various lipid species within subcellular compartments are linked to a variety of pathologies, the most well-known being Niemann-Pick disease.⁵ Direct imaging of the lipid and cholesterol compositions of various subcellular compartments without the use of fluorophores that may alter organization and intracellular trafficking⁶ would provide a valuable alternative perspective on native cell structure, and ultimately, function. Furthermore, three-dimensional imaging would enable organelles and intracellular processes to be resolved.

We previously used high resolution secondary ion mass spectrometry (SIMS), which we performed with a Cameca NanoSIMS 50, to image the distributions of cholesterol and sphingolipids on the surfaces of fibroblast cells.⁷⁻⁹ For analyses performed on this instrument, a focused cesium or oxygen primary ion beam is scanned over the sample. Monoatomic and diatomic secondary ions with up to five different mass-to-charge ratios (m/z) are collected in parallel at every pixel, producing images of the elemental or isotopic composition at the surface of the sample with a lateral resolution as good as 50 nm.^{10,11} In our approach, distinct stable isotopes that encode for component identity are selectively incorporated into the lipid species of interest with established metabolic labeling techniques.¹² Then, the component-specific isotope enrichment in the membrane cell is imaged with approximately 90 nm lateral resolution with a Cameca NanoSIMS 50 instrument. Use of this approach to visualize metabolically incorporated ¹⁸O-cholesterol and

¹⁵N-sphingolipids on the surfaces of fibroblast cells revealed the plasma membrane contained a fairly uniform cholesterol distribution and micrometer-scale ¹⁵N-sphingolipid patches.⁷ By imaging the effects of drugs on the sphingolipid distribution, we determined the sphingolipids were confined within domains by the cytoskeleton and its associated proteins.^{7,8}

Having identified the cholesterol and sphingolipid distribution within the plasma membrane, we have now turned our focus to investigating their intracellular distributions, including their abundances within intracellular membranes. The compositions of intracellular membranes have been previously assessed by lysing populations of cells, separating the organelles with centrifugation, and then analyzing the lipid composition in each fraction by biochemical assays or liquid chromatography-mass spectrometry (LC-MS).^{13,14} A pitfall of this approach is that the measured cholesterol levels depend on the method used to fractionate the cells, likely due to contamination with membranes from other organelles and spontaneous cholesterol transfer between membranes after cell disruption.¹⁵ SIMS is an attractive alternative approach for probing the distributions of lipids and metabolites within intact cells. A thin layer of material is sputtered from the sample each time the primary ion beam interrogates its surface, allowing a series of images to be acquired at progressively increasing depth below the cell surface by successively reimaging the same location multiple times. Sputtering scans that remove more material from the sample's surface are often inserted between the imaging scans to reduce the analysis time required to depth profile through the cells.^{16,17} Both NanoSIMS instruments and new time-of-flight SIMS (ToF-SIMS) instruments that do not need distinct labels for component identification have been used to image the intracellular distributions of nucleotides, lipids, amino acids, nanoparticles, and elements of interest.¹⁸⁻²⁴

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