

QUALITATIVE CHEMICAL ANALYSIS OF SUBCELLULAR DOMAINS WITH MULTI-ISOTOPE IMAGING MASS SPECTROMETRY (MIMS)

Martin Schwartz (maschwartz@virginia.edu)¹, Claude Lechene²

¹University of Virginia, Box 801394, Charlottesville, VA, 22908, USA

²Harvard Medical School and Brigham and Women's Hospital, 65 Landsdowne Street, Room 535, Cambridge, MA, 02139, USA

In a systematic study of the subcellular elemental chemical composition, we found domains showing distinct relative abundance of P, C and N on the endothelial cell surface. Cells were cultured on silicon supports, chemically fixed, dried and analyzed with MIMS (primary Cs⁺ ion beam, 16kV, 1pA). The mass images of the surface of the cells were recorded in parallel at mass ¹²C⁻, ¹²C¹⁴N⁻ and ³¹P⁻. The data were analyzed using overlay color-coding analysis. The results show that the area over the nucleus was relatively rich in nitrogen but poor in phosphorus, likely indicating that the nucleus was still covered by the cell membrane and/or the cytoplasm. The lamellipodia were relatively rich in phosphorus and very poor in nitrogen, likely reflecting the presence of phospholipids. The outmost edge of the cell was relatively rich in carbon and poor in nitrogen. Short filopodia had a relatively high nitrogen content, likely reflecting focal adhesion proteins.

After 'shaving' the top of the cell using an intense primary Cs⁺ ion beam, we were able to record a high ³¹P signal, particularly at the peri-nuclear membrane (likely the heterochromatin) and in two distinct structures inside the nucleus (likely the nucleoli). The nucleus was relatively rich in ¹⁴N compared to ¹²C. Phosphorus and ¹²C¹⁴N were colocalized at the nucleus periphery and in the nucleoli. There was a relatively small ¹²C content in the area of ¹²C¹⁴N and ³¹P colocalization. Areas relatively rich in ¹⁴N and poor in ³¹P may reflect the presence of a relatively large amount of nuclear proteins.

We made an intriguing observation. We have reanalyzed the cells, recording the masses of ¹H⁻ and ¹²C⁻ in parallel. At the peri-nuclear and nuclear level, we found micro-sized domains characterized by a large increase in the hydrogen signal and by a 50% relative decrease of the carbon signal. Such nuclear domains may be related to areas with high lipid concentration.

In conclusion, by taking advantage of MIMS parallel detection and without any isotopic addition, we have identified, for the first time, subcellular microdomains of particular elemental composition. This opens a new avenue to the study of cellular organization and function.

Supported in part by research resource grant 9P41 EB001974-04