

# MEASURE OF $^{15}\text{N}/^{14}\text{N}$ and $^{13}\text{C}/^{12}\text{C}$ ISOTOPE RATIOS IN CULTURED CELLS WITH MULTI-ISOTOPES IMAGING MASS SPECTROMETRY (MIMS)

Ralph Peteranderl (rpeteranderl@rics.bwh.harvard.edu)<sup>1</sup>, Claude Lechene<sup>1</sup>

<sup>1</sup>Harvard Medical School and Brigham and Women's Hospital, 65 Landsdowne Street, Cambridge, MA, 02139, USA

We used MIMS to measure the  $^{15}\text{N}/^{14}\text{N}$  and  $^{13}\text{C}/^{12}\text{C}$  isotope ratios in cultured cells from a rat embryo fibroblast cell line (REF 52) grown on silicon chips. Cells were cultured in DME/F12 medium with 10% FBS. Some of the cultures were supplemented with either  $^{15}\text{N}$  glycine or U- $^{13}\text{C}$  glycine. Prior to analysis, cells were either chemically fixed with a glutaraldehyde mixture (fix), or freeze-dried at  $-80^{\circ}\text{C}$  (fd)<sup>1</sup>. MIMS analysis was performed with a static  $\text{Cs}^+$  primary ion beam (16 keV;  $\approx 3\ \mu\text{m}$ ). The secondary ions were counted in parallel at mass  $^{12}\text{C}$ ,  $^{13}\text{C}$ ,  $^{12}\text{C}^{14}\text{N}$ , and  $^{12}\text{C}^{15}\text{N}$  (or  $^{13}\text{C}^{14}\text{N}$ ). Nitrogen isotope ratios were determined as  $^{12}\text{C}^{15}\text{N}^-/^{12}\text{C}^{14}\text{N}^-$ ; carbon ratios as  $^{13}\text{C}^-/^{12}\text{C}^-$  and as  $^{13}\text{C}^{14}\text{N}^-/^{12}\text{C}^{14}\text{N}^-$ .

The nitrogen isotope ratios determined for samples not supplemented with  $^{15}\text{N}$  glycine were 0.356% (fd) and 0.372% (fix), neither of which is significantly different from the terrestrial ratio (0.368%). The carbon isotope ratios for samples not supplemented with U- $^{13}\text{C}$  glycine were 1.071% (fd) and 1.131% (fix) when determined as  $^{13}\text{C}^-/^{12}\text{C}^-$ ; they were 1.092% (fd) and 1.159% (fix) when determined as  $^{13}\text{C}^{14}\text{N}^-/^{12}\text{C}^{14}\text{N}^-$ . None of these values is significantly different from the terrestrial ratio of 1.119%.

Labelling of the samples by supplementing the culture medium with 1 mM of  $^{15}\text{N}$ -glycine or 1mM of U- $^{13}\text{C}$ -glycine did lead to significant differences in the isotope ratios of the samples compared to the terrestrial ratios.  $^{15}\text{N}$ -glycine-labelled cells had nitrogen isotope ratios of 2.308% (fd) and 2.419% (fix). U- $^{13}\text{C}$ -glycine-labelled cells had  $^{13}\text{C}^-/^{12}\text{C}^-$  ratios of 1.385% (fd) and 1.326% (fix), and  $^{13}\text{C}^{14}\text{N}^-/^{12}\text{C}^{14}\text{N}^-$  ratios of 1.399% (fd) and 1.586% (fix), all of which are again significantly different from the terrestrial ratio (1.119%). The results demonstrate that  $^{13}\text{C}$  and  $^{15}\text{N}$  isotope ratios can be measured accurately in subcellular compartment. For the first time, this method opens the possibility to measure biomolecular turnover in intracellular compartments.

## References

1. Abraham, E. H., J. L. Brewslow, J. Epstein, P. Chang-Sing and C. Lechene., 1985. Preparation of individual human diploid fibroblasts and study of ion transport. *Am. J. Physiol. (Cell Physiol.)* 17: C154-C164

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