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Stable isotope probing coupled Raman microscopy: An efficient way to study single cell biochemistry

Hemanth Nag Noothalapati Venkata
Shimane University, Japan

Lipid droplets have been hypothesized to be intimately associated with intracellular proteins. However, there is little direct evidence for both spatiotemporal and functional relations between lipid droplets and proteins provided by molecular -level studies on intact cells. To elucidate the interplay between them at the single cell level, Raman microscopy was coupled with a very powerful strategy, namely, stable isotope labeling. Here, I present *in vivo* time lapse Raman imaging, coupled with stable-isotope (^{13}C) labeling, of single living *Schizosaccharomyces pombe* cells. Our results show that the proteins newly synthesized from incorporated ^{13}C-substrate are localized specifically to lipid droplets as the lipid concentration within the cell increases. Lipids, which help to store energy in a compact form, have variety of roles in biological systems and their metabolism is central to life. Here, we show that combination of stable isotope probing (SIP), Raman micro-spectroscopy and multivariate curve resolution analysis can serve as a valuable approach in metabolomics research. We studied ergosterol biosynthesis in single living fission yeast cells, grown in mixtures of normal (^{12}C) and ^{13}C-glucose as the sole carbon source. By carefully looking into the biosynthetic pathways and by comparing the observed peak positions with calculation results on isotope-substituted ergosterol, it is possible to understand how ^{13}C is incorporated in the conjugated C=C moiety of the molecule. The multivariate spectral data analysis revealed intrinsic spectra and their relative abundances of all isotopomers.

Biography

Hemanth Nag Noothalapati Venkata has completed his PhD from National Chiao Tung University, Taiwan. During his PhD, he studied spatio-temporal relationship between proteome and lipid droplet in single fission yeast cells *in vivo* by Raman microscopy. He has then developed methods to study single cell biochemistry utilizing carbon isotopes during his Post-doctorate at Ultimate Spectroscopy and Imaging Laboratory, NCTU. Later he moved to Shimane University, Japan as an Assistant Professor and has been actively working on medical and biological applications of Raman microspectroscopy.

nvhnag@life.shimane-u.ac.jp