Stable Isotope Labeling in Mammals (SILAM)

John R. Yates, III, PhD
Ernest W. Hahn Professor
The Scripps Research Institute, Chemical Physiology & Cell Biology, La Jolla, CA 92037 USA

As Stable Isotope Labeling with Amino Acids in Cell Culture (SILAC) is limited to cell culture, investigations at the tissue, organ or whole animal level require a different methodology. Stable Isotope Labeling in Mammals (SILAM) has been achieved utilizing ^{13}C as well as ^{15}N. Spirulina whole cells (lyophilized powder) (U-^{15}N, 98%+) (NLM-8401) have been used to uniformly label the proteome with ^{15}N. Animal models of human disease provide a powerful system for the study of molecular mechanisms associated with disease. A quantitative proteomic method for the study of *in vivo* biology using ^{15}N Stable Isotope Labeling in Mammals (SILAM) has been created through stable isotope labeling of the rats and mice.[1] Food is prepared using the algae *Arthrospira platensis* (also commonly called spirulina) grown on ^{15}N to incorporate the stable isotope into all proteins. By combining ^{15}N spirulina with a protein-free chow, food is created that provides only ^{15}N-labeled protein with the other nutrients and vitamins required for normal growth.[1-3] This method of stable isotope labeling uses the synthetic machinery of the cell to incorporate ^{15}N into proteins, and as a result it is a comprehensive technique for cell and tissue labeling. Labeled tissues can then be used as an internal standard when mixed with the diseased tissues from an animal model for a disease.

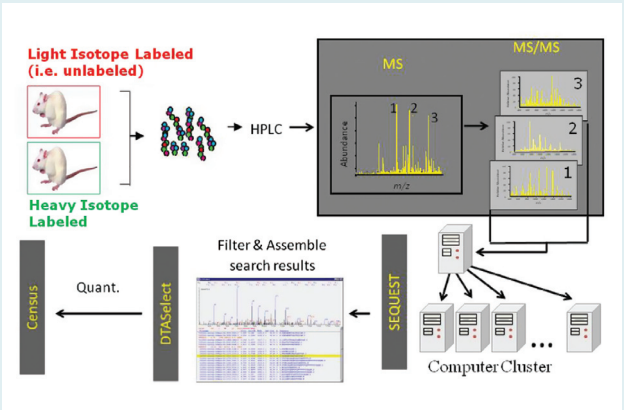

Figure 1. Overview of relative quantitation by stable isotope labeling and mass spectrometry.

By using "shotgun proteomics" (Figure 1), mixtures of the intact proteins are proteolytically digested and then analyzed by two-dimensional liquid chromatography coupled to a tandem mass spectrometer.[4] A tandem mass spectrometer can rapidly analyze peptides by generating fragmentation patterns for individual peptides in the mixture. Tandem mass spectra collected for peptides are then used as an "address" or "zip code" to identify proteins in sequence databases.[5] Peptides serve as a surrogate for the intact proteins and are used to identify a protein's presence, and through the stable isotope-labeling process, to measure changes in protein expression. By introducing ^{15}N-labeled amino acids into proteins, a "heavier" version of a protein is produced that can be readily differentiated from ^{14}N-labeled proteins (*e.g.* light) by a mass spectrometer. Thus, if a "heavy" normal mouse (control) is compared to a "light" diseased mouse, the differences in protein expression between the two can be determined using shotgun proteomics and mass spectrometry. This process will allow the discovery of pathways or processes that are up- or down-regulated as a function of disease. Other interesting experiments can be performed with SILAM such as "pulse-chase" in rats or mice to measure protein longevity – a parameter that may mark proteins as susceptible to damage and thus likely disease-related proteins.[6]

References

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