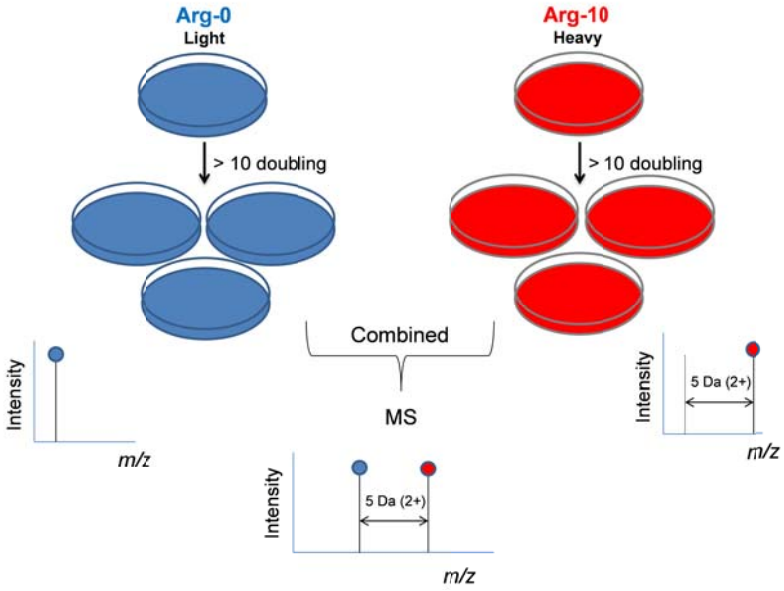
Quantification of protein expression by SILAC

As show illustrated in the below figure, **SILAC** (**stable isotope labeling with amino acids in cell culture**) is a technique based on mass spectrometry that detects differences in protein abundance among samples using the relative abundance between the two isotope differentially labeled peptide isoforms generated by digestion with a protease (ca, trypsin).[1][2][3][4] *



The principle of SILAC. Cells are differentially labeled by growing them in light medium with normal arginine (Arg-0, blue color) or medium with heavy arginine (Arg-10, red color). Metabolic incorporation of the amino acids into the proteins results in a mass shift of the corresponding peptides, for example, an increase of 5 Dalton for a 2+ peptide ion. This mass shift can be detected by a mass spectrometry as indicated by the depicted mass spectra. When both samples are combined, the ratio of peak intensities in the mass spectrum reflects the relative protein abundance. In this example, the labeled protein has the same abundance in both samples (ratio 1).

Procedure

Two populations of cells are cultivated in cell culture. One of the cell populations is fed with growth medium containing normal amino acids. In contrast, the second population is fed with growth medium containing amino acids labeled with stable (non-radioactive) heavy isotopes. For example, the medium can contain arginine ($^{13}C_6{}^{15}N_4$-R) labeled with six carbon-13 atoms ($^{13}C_6$) and four nitrogen-15 ($^{15}N_4$) instead of the normal carbon-12 (^{12}C) and nitrogen-14 (^{14}N). When the cells are growing in this medium, they incorporate the heavy arginine into all of their proteins. Thereafter, all peptides containing a single arginine are 10 Da heavier than their normal counterparts. The proteins from both cell populations can be combined and analyzed together by LC-MS/MS. Pairs of chemically identical peptides of different stable-isotope composition can be differentiated in a mass spectrometer owing to their mass difference. The ratio of peak intensities in the mass spectrum for such peptide pairs reflects the abundance ratio for the two proteins from which the peptides derived via digestion with a protease.

Applications

A SILAC approach involving incorporation of tyrosine labeled with nine carbon-13 atoms (^{13}C) instead of the normal carbon-12 (^{12}C) has been utilized to study tyrosine kinase substrates in signaling pathways.[5] SILAC has emerged as a very powerful method to study cell signaling, post translation modifications such as phosphorylation,[5][6] protein–protein interaction and regulation of gene expression. In addition, SILAC has become an important method in secretomics, the global study of secreted proteins and secretory pathways.[7] It can be used to distinguish between proteins secreted by cells in culture and serum contaminants.[8] Standardized protocols of SILAC for various applications have also been published.[9][10]

While SILAC had been mostly used in studying eukaryotic cells and cell cultures, it had been recently employed in bacteria and its multicellular biofilm in antibiotic tolerance, to differentiate tolerance and sensitive subpopulations.[11]

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