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SILAC Mouse: A novel tool for quantitative proteomics *in vivo*

MAX-PLANCK-GESELLSCHAFT

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Introduction

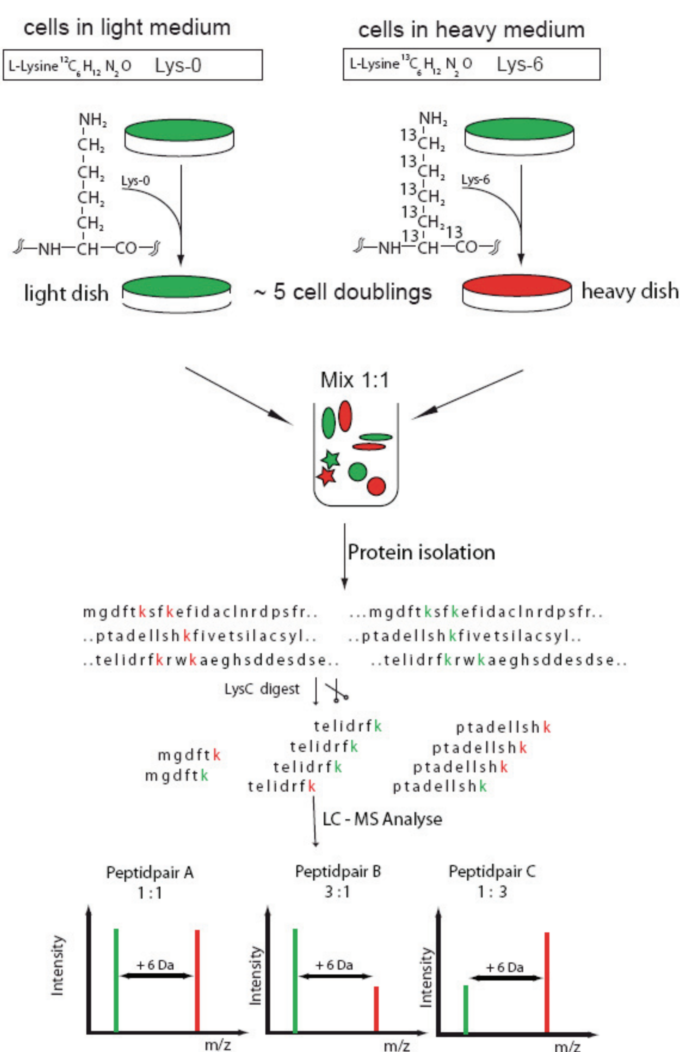
The administration of radioactive or stable isotope tracers to animals is a well-established technique for investigating the rate of protein synthesis, protein degradation, and flux in metabolic pathways *in vivo*. Stable isotopic labelling has been used for many decades. Even extensive incorporation of ^{13}C or ^{15}N stable isotopes does not result in discernible side effects.

Mass spectrometry (MS) is an inherently non-quantitative method, but relative changes in protein expression can be measured most accurately by comparing the natural form of a peptide with its stable isotopic analog. In recent years, ^{15}N labeling has been applied to microorganisms such as yeast, *C. elegans* and *Drosophila*. Even a rat has been partially or completely ^{15}N labeled. The SILAC technology has recently been introduced as an accurate tool for protein quantification. In a typical SILAC experiment, two cell populations are generated, one in a medium containing the natural amino acid (i.e. $^{12}C_6$-lysine) and the other in a medium containing the heavy isotope substituted version (i.e. $^{13}C_6$-lysine). This allows direct comparison of protein expression levels by mixing the non-labeled 'light' and labeled 'heavy' cell populations. Each peptide appears as a pair in MS analysis and the relative peak intensities reflect the abundance ratios. Here we investigated whether, and to what extent, whole mice can be labeled using the SILAC approach. Furthermore, we used *in vivo* SILAC to analyze the turnover of plasma and tissue proteins and the proteome of knock-out mice by state of the art proteomic methods.

1. The SILAC Method

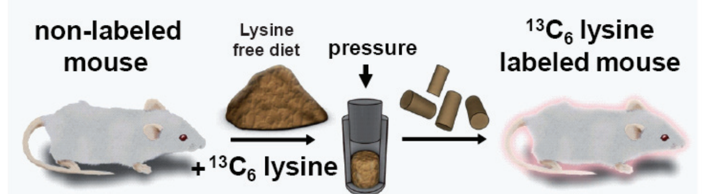
For the accurate quantification of proteins, the stable isotope labelling of amino acids in cell culture (SILAC) is a versatile tool. Briefly, in each experiment one cell population was grown in unlabeled medium as a control and a second population was grown in medium substituted with a heavy amino acid (i.e. Lys-6). Proteins of the two cell populations could then be distinguished during mass spectrometric analysis by a 6 Da (lysine-containing peptides) mass shift of their peaks. After complete incorporation of the heavy SILAC amino acids, the two proteomes were mixed in a 1:1 ratio. After protein digestion and GeLC-MS analysis peptides were quantified by peak intensities. The height of the light and heavy peak of a certain peptide is determined by MS analysis and the ratio is calculated by different software tools (i.e.MSQuant).

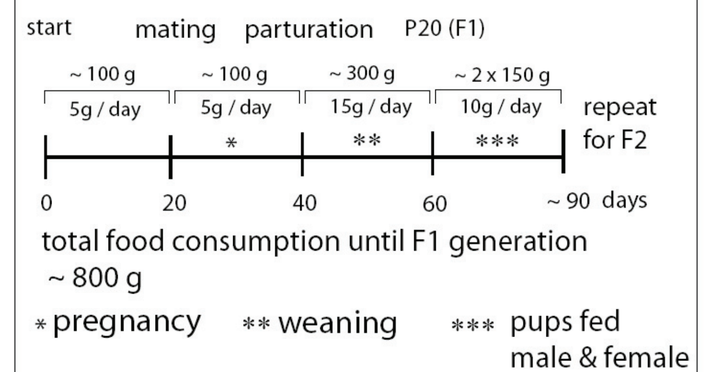
Stable isotope labeling of aminso acids in cell culture
(SILAC)



2. Mice feed production

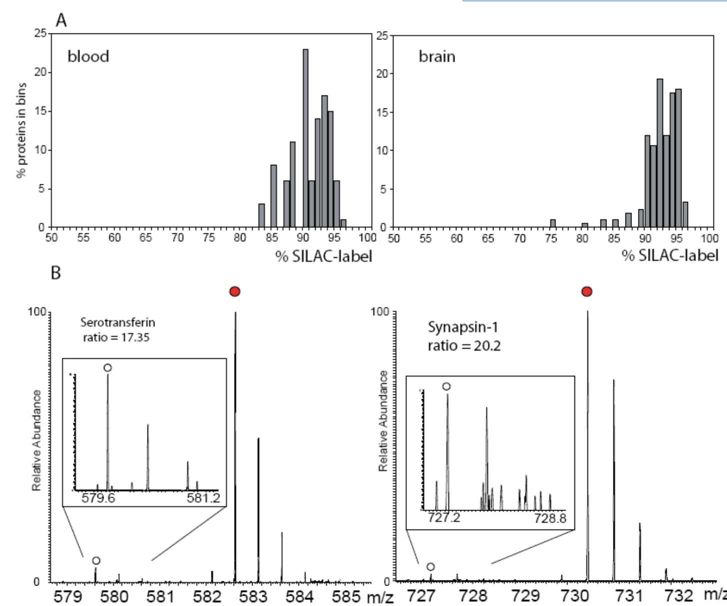
A lysine-free diet (from Harlan) was supplemented with normal ($^{12}C_6$) lysine or heavy ($^{13}C_6$) lysine compacted by pelleting and administered to mice. The consumption was 5-15 g per mouse/day. About 800g of each light and heavy feed was used during F1 generation. The feeding scheme is shown below.



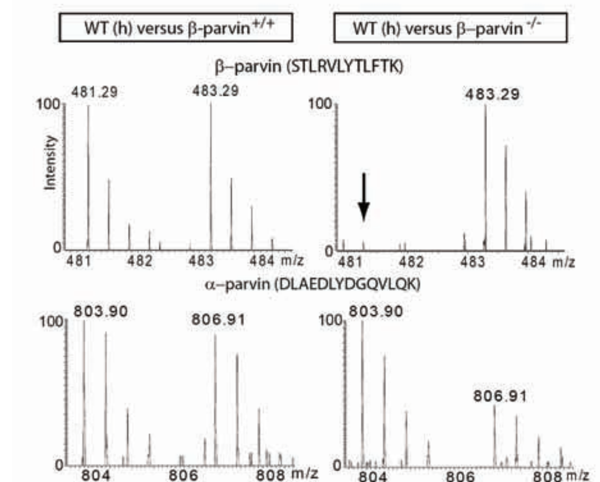


3. Label efficiency of the F1 generation in blood and brain.

(A) SILAC labeling of different blood and brain tissues of the F1 generation at P2. The average $^{13}C_6$-lysine incorporation is displayed in the histogram. (B) Selected mass spectra indicate peaks of light and heavy peptides.

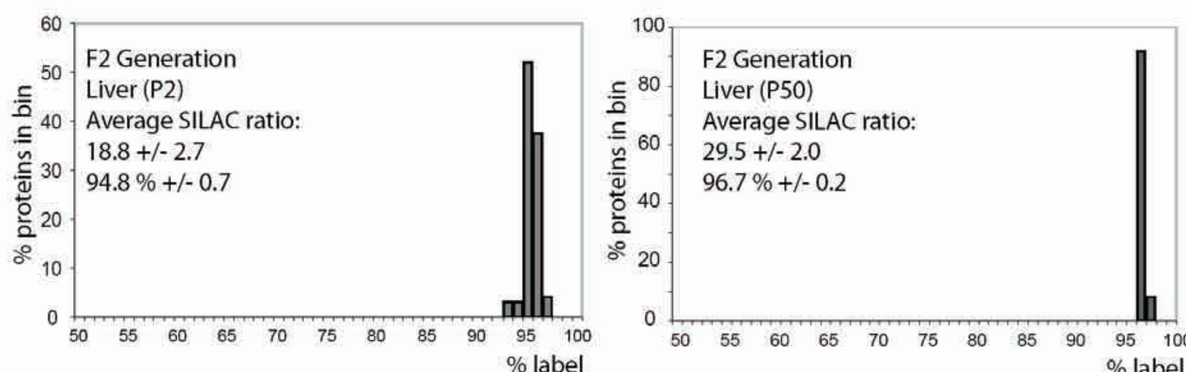


5. Heart tissue from SILAC labeled wild-type mice were mixed with non-labeled -parvin (+/+) heart and -parvin (-/-) heart (C). Selected peaks from alpha and beta parvin were shown.

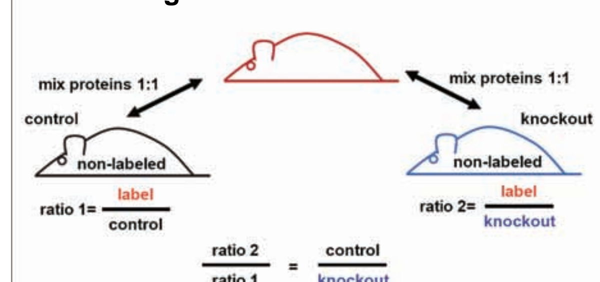


4. Lysine-6 incorporation of the F2 generation at P2 and P50.

The average label efficiency of the F2 generation is higher approx. 95%



6. Labelling scheme



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