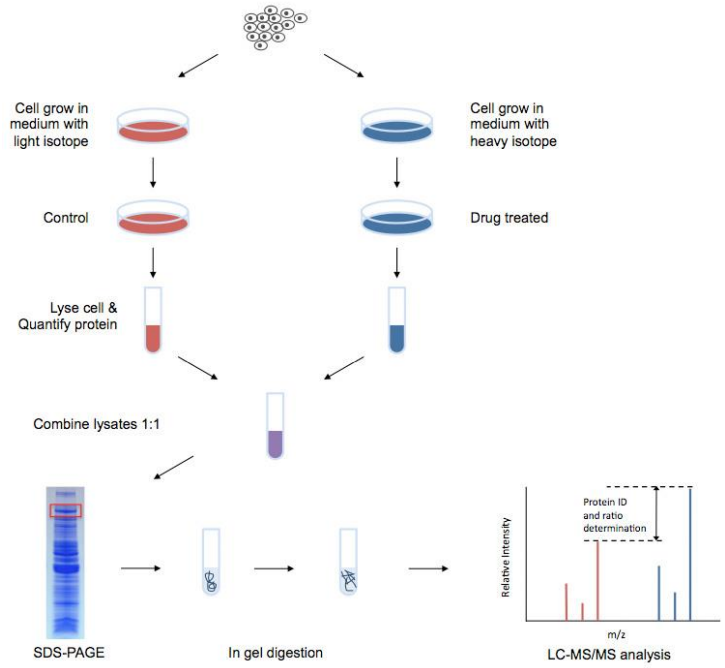
Quantitative Analysis of *In Vivo* Labeled Mitochondrial Protein

Mitochondrial ribosomes are specialized in the synthesis of several important inner membrane proteins of the respiratory chain. Stable isotope labeling by amino acids in cell culture (SILAC) is a labeling method that has almost no toxic side effects. Creative proteomics can provide SILAC-based marker quantification technology to accelerate your research on protein quantification, interactions, post-translational modifications, and new biomarkers.

SILAC-Based Quantitative Proteomic Analysis

Mutations in mitoribosomal proteins (MRPs), rRNAs, and assembly factors are responsible for a growing number of severe human disorders. In addition, mitochondria and their accessory proteins are increasingly recognized as cancer biomarkers and targets for cancer treatment. Therefore, it is important to understand how mitochondrial ribosomes are assembled. Protein quantification by SILAC combined with high-resolution mass spectrometry has become an important tool for quantitative proteomics research. Creative Proteomics provides SILAC-Based quantitative analysis technology to help analyze the relationship between mitochondrial matrix protein Mam33 and Saccharomyces cerevisiae mitoribosome biogenesis.

ASSAY OVERVIEW



Mitochondria prepared from mam33Δ cells (MHY2021) were grown to mid-log (OD600=1.2) in minimal galactose medium containing all amino acids. Wild-type cells (NB40-36a) were grown under identical conditions, except arginine and lysine were substituted with heavy isotopes. Light (mam33Δ) and heavy (WT) purified mitochondria are subjected to a series of processes such as centrifugation to extract their proteins. Proteins are then hydrolyzed using trypsin. Finally, extracted peptides were analyzed by Nano LC-MS / MS.

DATA OVERVIEW

Most large and small subunit ribosomal proteins remained stable, while Mrp20 was decreased (62% of WT). Of all the proteins to which Mam33 directly binds, only MrpL27 was destabilized in mam33Δ cells (mL41; 48% of WT). In addition, MrpL13 (mL50; 69% of WT) and MrpL38 (uL14; 46% of WT) were decreased. Mam33 can prevent misfolding and aggregation but has a minimal effect on MRP steady-state levels.

Features

- Nano LC is performed using an Ultimate3000 nano UHPLC with a 100 μm x 10 cm in-house made nanocolumn packed with a reversed-phase ReproSil-Pur C18-AQ resin.
- Mass spectrometry is performed using a Q Exactive HF mass spectrometer.
- High sensitivity
- Suitable for studying protein-protein interaction
- Quick turnaround time
- Fully automatic, high-throughput, one-stop protein quantification service

Applications

- Study the interaction of proteins with DNA, RNA, proteasomes, and small molecule drugs
- Study the post-translational modification of proteins
- Find new biomarkers

Reference

Hillman G A, Henry M F. The yeast protein Mam33 functions in the assembly of the mitochondrial ribosome. *Journal of Biological Chemistry*, 2019, 294(25): 9813-9829.

Contact Us

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