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Review article

Mitochondrial protein turnover: Methods to measure turnover rates on a large scale

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ABSTRACT

Mitochondrial proteins carry out diverse cellular functions including ATP synthesis, ion homeostasis, cell death signaling, and fatty acid metabolism and biogenesis. Compromised mitochondrial quality control is implicated in various human disorders including cardiac diseases. Recently it has emerged that mitochondrial protein turnover can serve as an informative cellular parameter to characterize mitochondrial quality and uncover disease mechanisms. The turnover rate of a mitochondrial protein reflects its homeostasis and dynamics under the quality control systems acting on mitochondria at a particular cell state. This review article summarizes some recent advances and outstanding challenges for measuring the turnover rates of mitochondrial proteins in health and disease. This article is part of a Special Issue entitled "Mitochondria: From Basic Mitochondrial Biology to Cardiovascular Disease".

Contents

1. Introduction

Proteins in the cell exist in a dynamic equilibrium of continual synthesis and degradation. Alterations of this equilibrium are often reflected in changes in static protein abundance, which is commonly measured to determine the involvement of particular proteins in disease mechanisms. Static abundance alone however lacks temporality and gives little insight into the window of impact or homeostasis of a protein. Protein turnover rate is thought to provide this "missing dimension" of protein function [1], and has drawn particular attention in mitochondrial biology due to the importance of mitochondrial dynamics and quality control in human diseases and aging. The proximity of mitochondrial proteins to reactive oxygen species renders them highly susceptible to protein damage. Given the importance of mitochondrial

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proteins to numerous cardiac processes, it is essential that the renewal, or turnover, of mitochondrial proteins be sustained during elevated stress conditions. Maintenance of mitochondrial quality and integrity requires coordinated turnover of compromised components to preserve cellular functions, whereas the accumulation of protein damage is thought to contribute to disease pathology [2,3].

There has been a longstanding interest in biomedical research to examine the rates and permutations of protein turnover in the past eighty years. Early experiments were pioneered by Rudolf Schoenheimer and David Rittenberg at Columbia University [4–7], who in 1939 used isotope labeling to establish that proteins in the body, and thus life itself, are in a dynamic state of interactions with the environment even in the absence of obvious growth [8]. These tenets ran contrary to popular beliefs of the day, which posited that organic molecules acquired through diet were solely burnt for fuel, but they soon gained wide acceptance and are now held as self-evident. Studies from the 1950's onward coincided with interests in measuring the flux of biochemical pathways, and led to the elucidation of the bulk protein synthesis rate in the human body [9] and of the rate constant of turnover (k) of specific cellular compartments, and a few easily isolatable proteins [10].

In cardiac biology, Murray Rabinowitz at the University of Chicago was among the first to realize important links between mitochondrial protein turnover and hypertrophy [11,12]. In 1973, he observed that following aortic banding, rat heart cytochrome c abundance increased on the first day, then subsequently decreased, whereas its degradation decreased consistently throughout (as measured by pulse-chase of a radioactive heme precursor) [13,14]. He concluded that the decreased degradation of cytochrome c was responsible for its early increase in hypertrophy, and that dissociation in myofibrillar and mitochondrial growth responses ultimately led to energetic decline. Meanwhile, other groups began to explore the turnover of mitochondrial sub-compartments (outer membrane vs. inner membrane) or few individual mitochondrial proteins such as cytochromes [10,15,16]. Two observations from these early studies are of particular interest: (i) they showcased the idea that protein turnover is a regulated cellular parameter that responds to disease stimuli; (ii) it was realized that individual mitochondrial compartments and proteins exhibit different

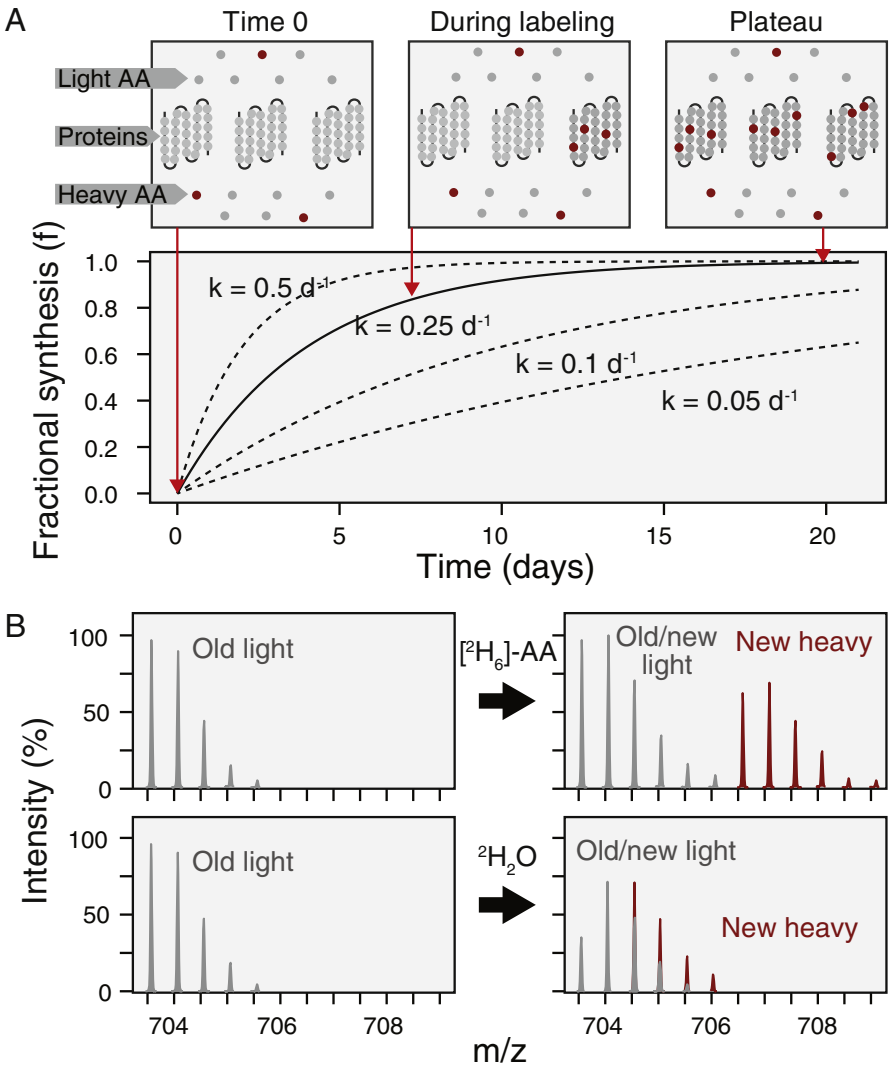

Fig. 1. Determination of protein turnover rates with stable isotope labeling. A. (Upper left): At time 0 of the labeling experiment, some cellular amino acids (gray circles) are labeled with heavy isotopes (red circles). (Upper middle): During the course of the labeling experiment, as old proteins are degraded and new proteins are made, a fixed amount of heavy isotopes becomes incorporated into the protein pool. (Upper right): Eventually, the protein pool reaches steady state as all protein molecules contain a fixed ratio of isotopes. (Lower panel): Incorporation rate of stable isotope labels corresponds to the portion of new proteins in the biological systems, which can be used to calculate the turnover rate of the proteins. The first-order kinetic curves corresponding to a protein with multiple hypothetical turnover rates (k = 0.05, 0.1, 0.25, or 0.5 d^{-1}) are shown. B. The analytical approach to quantify the amount of heavy isotope labels with mass spectrometry at any given time is dependent on the type of labels used. (Upper): Stable isotope labeled amino acid approaches create a new peptide isotopomer envelope in the mass spectrum that corresponds with newly made peptides with heavy labels. (Lower): Incorporation of ^2H labels from ^2H_2O results in a shift in the fractional abundance of isotopomers in the envelope.