Published in final edited form as:

J Mol Cell Cardiol. 2015 January ; 78: 54–61. doi:10.1016/j.yjmcc.2014.10.012.

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

X'avia CY Chan¹, Caitlin M Black¹, Amanda J Lin¹, Peipei Ping^{1,2}, and Edward Lau^{1,*}

¹The NHLBI Proteomics Center at UCLA, Department of Physiology¹, UCLA David Geffen School of Medicine, Los Angeles, CA 90095, USA

²Department of Medicine, and Bioinformatics², UCLA David Geffen School of Medicine, Los Angeles, CA 90095, USA

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.

Keywords

Mitochondria; protein turnover; proteome dynamics; proteomics; heavy water

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

*Correspondence should be addressed to Edward Lau (edwardlau@ucla.edu). Additional correspondence to Peipei Ping (pping@mednet.ucla.edu), 675 Charles E Young Drive S 1-619, University of California at Los Angeles, Los Angeles, CA 90095, USA, +1 (310) 267-5624.

Disclosures

None.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

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 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 turnover rates. However, these studies largely lacked the technical prowess to discern individual protein turnover on a large scale, and the ponderous experimental design and large variability of the results both hindered examination of disease models until modern proteomics techniques became available.

2. Methods for proteome-wide turnover studies: from single cells to whole animals

To measure the physiological protein turnover rate constant, i.e., the rate at which existing proteins are replaced, it follows that a method is needed to differentiate a newly synthesized protein from pre-existing proteins after the protein sample is drawn from the living organism (Figure 1A). Since 1935, isotope-labeling methods have been widely employed to label cellular proteins and trace their turnover. Deuterium was among the earliest labels used as an

analog for biomolecules, when Schoenheimer explored protein metabolism using molecules tagged with ^2H and ^{15}N . However, the laborious procedures to discern stable isotope contents (with interferometry or the Kjeldahl method) led to their gradual displacement by the advent of radioisotope analogs such as ^3H or ^{14}C -labeled amino acids in the 1940s, which were easy to detect using scintillation counters. With the availability of advanced mass spectrometry techniques in the late 1990s, there has been a marked return to the use of stable isotope labels, which can be easily resolved by their mass. The advantages of stable isotope mass spectrometry over radioisotope approaches include its safety, bio-orthogonality, the ability to quantify multiple labeled proteins without isolation, and the ability to resolve a peptide cluster with multiple numbers of labels, which allows the determination of the fraction of new proteins in a system through precursor-product relationship (see below).

As protein analytical techniques mature to enable the analysis of minute amounts of individual protein species, the focus of protein turnover studies has changed in parallel and has steadily gained in finesse and sophistication. Whilst early studies focused on the measurements of bulk body protein turnover, scientists eventually began to measure the turnover of whole mitochondria, mitochondrial sub-compartments [17], or the turnover of highly abundant proteins that were relatively easy to isolate in bulk [18,19]. The development of modern proteomics techniques has spurred a leap in capability, allowing investigators to go from monitoring a few proteins in one study to measuring the individual turnover rates of hundreds or thousands of proteins simultaneously.

Proteome-wide investigations of turnover rates were first demonstrated in single cell systems in vitro. A number of mature techniques current exist that allows protein turnover or stability to be measured in cultured cells, including both isotope-based as well as some non-isotope-based approaches. Non-isotope-based approaches include the use of immunobiological assays to measure protein abundance following synthesis inhibition (e.g., by cyclohexamide), and the measurement of the decay of protein fluorescence signals from fluorescent protein timers [20,21]. A common stable isotope approach suitable for in vitro studies is the use of dynamic or non-saturating stable isotope labeling by amino acids in cell culture (SILAC) [22,23]. SILAC was originally devised as a means to quantify relative protein abundance, by introducing fully heavy-labeled amino acids, typically arginine and lysine, in the culturing medium such that the cells produce isotope-labeled protein standards for comparison with cells grown in light medium. By measuring the rate of incorporation of the labels into proteins in the heavy-medium grown cells (or removal of labels in chase experiments), the method has been adapted to measure protein turnover. Dynamic SILAC-based experiments have been applied to different cultured cells including in bacteria [24,25], yeast [1] and multiple mammalian cell lines including HeLa, A549 human adenocarcinoma, and C2C12 mouse myoblasts [26–28]. These large-scale experiments have been transformative in defining the basic cell biological principles of protein turnover; e.g., the data revealed that protein complexes such as proteasomes often exhibit synchronized turnover [27]; that housekeeping proteins generally have slow turnover, whereas stress responsive elements such as heat shock proteins have faster turnover; and that protein turnover in part reconciles the discrepancies between mRNA and protein levels in a system [25].

Nevertheless, beginning with the first proteome wide investigation in animals in 2005 [29], interests are gradually building towards understanding in vivo protein turnover in animal disease models as opposed to that in individual cells. Just as many physiological processes are not easily reproduced in single cell systems, there are reasons to believe that the proteome dynamics of exponentially growing cells in vitro does not recapitulate the physiological regulations that occur in multicellular organisms. Direct comparisons between in vitro and in vivo turnover rates are challenging, in part because most in vitro experiments have been performed on transformed cell lines that do not have direct in vivo equivalents. What is known, however is that protein turnover rates are highly dependent on cell types, and likewise identical proteins from the mitochondria of different tissues of origin can have different turnover rates (see below). More importantly, cultured cells are not under the same protein degradation stress as cells in multicellular organisms, as cultured cells may turn over unwanted proteins both via degradation or dilution into daughter cells, which clearly would confound inference of physiological turnover rates. The minimum turnover rates of proteins are further limited by the doubling time of cells, in vitro experiments typically report mammalian protein half-life of hours (as opposed to days or weeks in whole organisms).

3. Stable isotope labels for proteome-wide turnover studies in animals

Hence, recent studies on mitochondrial turnover are turning toward proteome-wide analyses of individual turnover in whole animals to describe systems-level regulatory behaviors of the entire mitochondria in vivo. A challenge of deducing turnover in complex organisms is that the isotopes need to be effectively delivered into the organisms via infusion or by oral intake via food or water. In cell systems, the heavy isotopes can be expected to enrich to unity, such that no light precursor exists and all newly synthesized proteins carry only the heavy signature. In whole animals, however, the protein precursor cannot in practice be enriched fully within the experimental timeframe, due to dilution by abundant unlabeled precursors in the animal's biomass, compartmentalization, and potential toxicity. Three major types of isotope tracers are available for large-scale animal studies:

3.1. Stable isotope-labeling of essential amino acids

A popular method is to introduce of amino acids labeled with heavy isotopes (^{13}C , ^2H , or ^{15}N), such as $[\text{}^2\text{H}_3]$ -leucine, into the animal, either through injection or through an enriched synthetic diet. A major advantage of labeled amino acids is the simplicity in data analysis workflow. Since these heavy amino acids have fixed number of labels, no intermediates in the amount of labeling per newly synthesized protein exist. Secondly, precursors with +6 or +8 Da (e.g., $[\text{}^{13}\text{C}_6]$ -arginine $[\text{}^2\text{H}_8]$ -valine) are available, which are sufficiently different in mass from the light variant such that isotope clusters do not overlap (Figure 1B). A drawback is the requirement for a synthetic diet supplemented with the labeled amino acid, which may unbalance metabolism or spur protein synthesis. Labeled amino acids were first exploited by Beynon and colleagues to deduce the first proteome-wide protein turnover study in higher organisms [29], and have been employed in a number of studies in diverse animals including mouse [30,31], chicken [29], zebrafish [32], and carp [33]. Using $[\text{}^2\text{H}_3]$ -leucine, the Rabinovitch group recently determined the in vivo turnover rates of ~400 mouse liver and heart mitochondrial proteins [31].

3.2. Metabolic ^{15}N or ^{13}C labeling via diet

Alternatively, proteins can be enriched via metabolic precursors. Enrichment of ^{15}N atoms in an animal has been achieved for SILAC mice (SILAM), a method for creating >95% ^{15}N -labeled mice to be used as internal standards for protein quantitation, where the isotopically pure foodstuff for animals labels all mouse amino acids metabolically. Similar to dynamic SILAC experiments, a non-saturated labeling curve can be used to measure protein turnover in live animals. This method is compatible with proteome-wide turnover inquiry [34,35], and has recently been demonstrated for the measurement of turnover rates of over 1,700 proteins in the brain, liver, and blood of mice fed with the ^{15}N -enriched *spirulina* algae for up to 32 days [35]. Besides ^{15}N , metabolic ^{13}C labeling has also been demonstrated by enriching mouse diets with ^{13}C -labeled glucose, which is metabolized into amino acid precursors [36].

One drawback of metabolic ^{15}N or ^{13}C labeling is the complex isotope patterns generated as compared to SILAC approaches. As the mice gradually acquire excess heavy atoms, the labeled peptide peaks will shift gradually both in relative abundance of heavy isotopes (due to protein turnover) and in the amount of horizontal mass shifts exhibited by the heavy labeled peptides (due to increasing numbers of heavy atoms in newly synthesized proteins), creating a complex pattern of isotopic shifts [37,38] which demands more complicated data processing to deconvolute the spectra into component peptide ions with different numbers of incorporated heavy atoms.

3.3 Metabolic labeling with heavy water

$^2\text{H}_2\text{O}$ (deuterated heavy water) is gaining in popularity as a protein label for animal studies [39–41]. One primary advantage of $^2\text{H}_2\text{O}$ is that it can be straightforwardly introduced into the animal by free intake from the drinking water supply, which avoids potential physiological impacts of dietary modifications or amino acid infusion. The ingested $^2\text{H}_2\text{O}$ molecules quickly equilibrate with body water, thus precursor isotope enrichment can be measured accurately from any biofluid. Deuterium atoms from body water is conferred to the carbon-hydrogen bonds of free non-essential amino acids during their enzymatic biosynthesis or via transaminases [42], which is shown to complete within 30 minutes [43]. Unlike in deuterium exchange experiments where solvent-exchangeable amide bonds are labeled, the enzymatically labeled sites are primarily chemically stable C-H bonds and do not back-exchange during sample processing. $^2\text{H}_2\text{O}$ labeling does not create separate peptide clusters (which essentially doubles the complexity of the proteomics sample), and thus is very amenable to large-scale analysis. Recently $^2\text{H}_2\text{O}$ labeling has been demonstrated to quantify the turnover rates of over 2,900 proteins in the mouse heart. The method has been used for proteome-wide turnover measurements in multiple organisms including the mouse [44,45], rat [46,47], and human [41,48]. A potential drawback is that deuterated peptides elute slightly earlier in liquid chromatography, which may introduce errors in peak area quantification. Secondly, the number of ^2H accessible labeling sites may be uncertain in some scenarios, such as in systems with different biochemistry of amino acid utilization than mammals, or in peptides with post-translational modifications.

As another isotope analog of water, H_2^{18}O shares many similarities with $^2\text{H}_2\text{O}$ in labeling characteristics and operational ease. Unlike $^2\text{H}_2\text{O}$, H_2^{18}O labels the carboxyl oxygen atoms of amino acids, thus allowing easy ascertainment of the number of labeling sites on a peptide. It is also thought to circumvent the label reutilization. Since labeling occurs upon the cleavage of a peptide bond or from the amino acyl t-RNA linkage [49], any proteolytic amino acids will subsequently become labeled with ^{18}O when they are re-incorporated into peptide bonds. Stephen Previs and colleagues compared the protein turnover rate of serum albumin in mice using both $^2\text{H}_2\text{O}$ and H_2^{18}O methods [50], and found that H_2^{18}O labeling returned faster turnover rates, although the difference did not reach significance ($0.325 \pm 0.046 \text{ d}^{-1}$ for $^2\text{H}_2\text{O}$ labeling versus $0.301 \pm 0.039 \text{ d}^{-1}$ for H_2^{18}O labeling, $P = 0.17$). Since H_2^{18}O labels fewer atoms than $^2\text{H}_2\text{O}$ per peptide, newly synthesized proteins are more difficult to discern and at present it is not commonly used for large-scale studies.

4. Analytical and computational approaches to deduce turnover rates

The optimal isotope label for an experiment depends greatly on the available analytical instrumentation and computational workflows. Economy, physiological impacts, and ease of data analysis are all valid concerns that may influence decision as has been reviewed elsewhere [51]. Experiments with different labels have generally returned comparable turnover rates of mitochondrial proteins (See Table 1). In all of the above stable labeling experiments, the incorporated labels may be detected by mass spectrometry. Most modern proteomics platforms have been successfully employed for protein turnover studies, including Orbitrap [39,40], Q-ToF [41], MALDI-ToF [29], and LTQ-FT [31,35] instruments.

The major analytical challenge in a protein turnover experiment is to determine the amount of labels in unlabeled, partially labeled, and fully labeled samples in order to deduce the rate of label incorporation. As discussed above, in animal studies a low and uncertain level of enrichment is typically achieved. Converting the amount of labels present to the fraction of new proteins thus presents a challenge when the true precursor isotope enrichment is difficult to ascertain, since it creates an uncertainty in how the fully labeled molecule may resemble in terms of relative isotope abundance (analogous to specific activity in radioisotope labeling), which is needed to deduce the fraction of newly-synthesized product. A general solution to deduce protein synthesis from isotope labels is to assume a combinatorial probability model, which dictates that a biological polymer of more than one monomer (such as a protein) will exhibit a statistical distribution of labels that follow the polynomial expansion of the proportion of labeled and unlabeled precursors. This was exploited in 1986 by Kalderon and colleagues to measure glycogen synthesis from ^{13}C -labeled glucose [52], and by Marc Hellerstein and colleagues in 1991, who put forth an application of the technique to mass spectrometry data, named Mass Isotopomer Distribution Analysis (MIDA) (Reviewed in [53]). Mass isotopomers are isomers containing different isotopes that become resolved by a mass analyzer. Thus as a protein is synthesized by a population of monomers (protein precursor) containing a fixed proportion of heavy isotopes, the resulting statistical distribution of protein isotopomers containing zero, one, two, or more heavier isotopes will follow a mathematically deducible polynomial distributions determined by three MIDA parameters: the number of monomer units in the

protein, the proportion of labels in the precursor, and the fraction of newly made polymers (whose amounts of labels are diluted by the presence of unlabeled polymers in the pool). The feasibility of this method for calculating protein turnover was first demonstrated on serum albumin in [$^2\text{H}_3$]-leucine labeled rats [54]. Variations of the combinatorial probability model have since been widely used to analyze MS data in protein turnover experiments. For instance, Robert Beynon and colleagues devised a method to identify the precursor relative isotope abundance in single amino acid labels (e.g., [$^2\text{H}_8$]-valine) that uses the combinatorial probability model to compare the isotopomer distributions of peptide clusters containing two instances of the labeled amino acid, in order to deduce the true precursor enrichment, which is then used to calculate the fraction of newly synthesized peptides for all peptides containing at least one instance of the labeled amino acid. In combination with high-throughput proteomics techniques, this approach led to the first large-scale measurements of individual protein half-life in vivo in higher organisms in 2005 [29].

Protein turnover is assumed to be a first-order kinetic process. As the heavy labels are integrated into the newly synthesized proteins, with eventual turnover of the protein pool the amount of label will reach steady-state equilibrium with the surrounding. As the fraction of newly synthesized proteins are experimentally determined at one or more time point, the data could be fitted to a kinetic model. Multiple models have been proposed. In most cases, simple rise-to-plateau kinetics is assumed, and the isotope distribution before labeling and at steady-state can be calculated as above determine the remaining unknown (the protein turnover rate) [51], or alternatively all three parameters can be found using multi-parameter fitting [44]. Variations to this first-order kinetics model were described. Takhar Kasumov and colleagues have used the quasi-linear region of the first-order kinetic curve to calculate k for long-half-life proteins [55]. Our group has described a model that assumes separate protein and precursor rate constants with detailed methods in Lam et al. [40]. More complex multi-compartment modeling has also been formulated [56].

Although the isotope labeled spectra can be manually analyzed to yield turnover rates, high-throughput studies demand the aid of computational software automation. Many existing tools can integrate the peak areas of native or isotope-labeled peptide ions for protein quantification process. Since these software applications must add together the relative peak areas of each isotope, they may be coopted for protein turnover studies when a custom kinetic model is supplied to handle the integration data.

Several software applications have been written explicitly for protein turnover analysis, each of which is targeted for specific applications. **(i)** SILACTor: Developed by James Bruce and colleagues at the University of Washington, it is a command line software tool written to facilitate the analysis of dynamic SILAC experiments in cell culture. An advantage of SILACTor lies in its simplicity, as the software assumes that the heavy precursor is enriched to unity, such that protein labels go from all light peptides to all heavy peptides and vice versa, as is attainable in in vitro experiments. The relative isotope abundance of a peptide time series at each time point is collated and fitted to a kinetic curve to output. The software additionally has the capability to quantify peptide ions that do not have specific database identifications through an accurate mass retention time inclusion list to increase data depth. SILACTor is not compatible with partial enrichment such as in larger animals. **(ii)**

Topograph: Topograph is a Windows application developed by Michael MacCoss and colleagues at the University of Washington. Topograph (version 2) is compatible with ^{15}N and isotope-labeled amino acids, and is suitable for both in vitro and in vivo animal studies [31], by automating the method of using the spectra of multiply-labeled peptides to deduce precursor RIA first proposed by Beynon. Topograph also contains a user interface that can generate visual output, as well as advanced options including to account for earlier elution of deuterated peptides. (iii) ProTurn: ProTurn is a cross-platform software application specifically written by our group specifically for the analysis $^2\text{H}_2\text{O}$ labeling experiment data. ProTurn (version 2.0.5) features a user interface which allows the users to specify protein identification output from a number of search engines (e.g., ProLuCID [57], Thermo Proteome Discoverer, and COPaKB [58]), options for peak integration mass window and multiple data smoothing types. Users can also visualize and compare results in the interface. A general limitation of the above tools is that at present they remain standalone specialty applications, requiring users to adapt to their data analysis workflows and perform multiple file format conversions to the specifications of software input/output. It is thought that with improvements to data sharing policy and software crosstalk and federation, future analytical tools will more tightly integrate with existing workflows on data acquisition and protein identification.

5. General features of mitochondrial proteome dynamics

The scope and coverage of protein turnover experiments are fast approaching that of quantitative protein expression profiling. In a recent experimental dataset in mouse heart, liver, and kidney by our group (Peipei Ping laboratory), we measured the turnover rates of 694 out of the total of 1,088 (64%) mouse proteins that are annotated as mitochondrial proteins on SwissProt/PIR. Several properties of mitochondrial proteome dynamics can be inferred from these large-scale studies. For instance, mitochondrial proteins exhibit a dynamic range of turnover rates even at basal level, which covers up to three orders of magnitude. Subunits of multi-protein complexes have more similar turnover rates, but individual protein variability exists that may be explained by sub-complex association [44]. There does not appear to be a significant difference in mitochondrial protein turnover when measured from purified mitochondrial samples and in whole-cell lysate, indicating import is probably not a bottleneck to the measured turnover rates [40]. The turnover rates of mitochondrial proteins differ by the sampled tissue, with the approximate order being that of blood > liver ~ kidney > heart > brain ~ skeletal muscle among the investigated tissues in mice (Table 1). Mitochondrial subpopulations in the heart (interfibrillar and subsarcolemmal mitochondria) can show different turnover rates, with subsarcolemmal proteins turning over faster [47]. Furthermore, identical protein species can exhibit very different turnover behavior relative to the whole mitochondrial sub-proteome (Figure 2). Differences in individual protein turnover do not appear to be governed by simple biophysical parameters (protein hydrophobicity, molecular weight, or isoelectric point) or sequence features. PEST motifs [59] and sequence disorders [60] have both been proposed to govern protein stability, but their presence has generally not been found to correlate with protein turnover rates in large-scale analyses.

Large-scale studies of protein turnover in animals are also enabling the examination of changing protein dynamics under various physiological and pathological processes. Our group measured the proteome-wide turnover of an animal heart disease model via isoproterenol challenge in mice, whereas William Stanley and colleagues examined the changes in interfibrillar and subsarcolemmal mitochondrial protein turnover in a transverse aortic constriction model. Hellerstein and colleagues found slower turnover liver of mitochondrial proteins in long-term caloric restriction in mice [45]; Peter Rabinovitch and colleagues examined the effect of aging and short-term caloric restriction [61]. A common feature of many such investigations is the complex, bidirectional changes in mitochondrial components that cannot be captured from the median of all mitochondrial proteins. The turnover rates of subsarcolemmal and interfibrillar mitochondrial proteins appear to permute independently in disease [46]. Protein subunits within a stable complex such as respiratory complex I can likewise respond to the same stimulus differently [40].

These findings emphasize the importance of finer details in individual protein homeostasis, whether orchestrated by intra-mitochondrial proteases [62], mitochondria-derived vesicles [63], or a combination thereof. Therefore although measurements of bulk mitochondrial turnover continue to be performed, with a number of recent studies of total mitochondrial protein turnover in models of caloric restrictions [64–66], the finding of widely varied turnover of mitochondrial proteins suggests that additional biological details may be revealed in proteome-scale studies. The measurements of proteome-wide dynamic ranges in whole animals will be preferred if finer regulatory details are to be elucidated.

6. Translational potential of proteome dynamics studies

Protein turnover rates can reveal novel protein markers with altered kinetics in human diseases, which may not be discernible from steady-state expression measurements. The potential of this approach has been demonstrated in pilot studies of patients with psoriasis and neurodegenerative diseases [67,68]. Labeling of human subjects is associated with additional challenges, for example: **(i)** The potential physiological impacts and side effects of the label need to be rigorously documented prior to applications to patients. **(ii)** The slower metabolism and protein turnover in human than smaller mammals requires longer labeling period or more stringent accurate measurements of minute degrees of label changes. **(iii)** As the subjects take in labels gradually, the data analytical workflow must be able to account for time-dependent precursor relative abundance in its kinetic models.

Several recent studies described the proteome-wide measurements of plasma turnover rates in human subjects using $^2\text{H}_2\text{O}$ labeling [40,41,48]. Hellerstein and colleagues measured the half-life of 100 proteins in four subjects, measuring precursor enrichment at multiple time points to adjust for label intake; whereas our group documented the labeling protocol and post-labeling monitoring in 10 subjects for up to 240 days and found no changes in hemodynamic behaviors and no signs of general discomfort among the subjects [48]. Several advantages of $^2\text{H}_2\text{O}$ labeling make it particularly applicable to human investigations; for instance, labeling without dietary modifications alleviates some difficulties in subject recruitment and enforcement of compliance to labeling regimens. $^2\text{H}_2\text{O}$ has an extensive and well-documented safety record in humans [41,69], and

no physiological effects have been reported at experimental dosages (~2%). Label enrichment can be accurately monitored from any body fluid. Lastly, $^2\text{H}_2\text{O}$ remains financially feasible within the scale of human consumption. A typical labeling regiment of 2% enrichment over 14 days involves the total intake of 0.75 to 1.5 L of 70% $^2\text{H}_2\text{O}$ and costs ~\$500 per subject at the time of writing. By contrast, equivalent isotope enrichment with ^{13}C -labeled leucine would be five to ten fold higher based on 3 g of total leucine intake per diem.

Some additional issues remain to be addressed before the measurements of mitochondrial turnover rates in human subjects in vivo can be conducted routinely, not least of all is the logistics of acquiring human mitochondrial samples. Cardiac samples are particularly limited in surgical availability, and repeated biopsy samples at multiple time points are not possible in most scenarios. A viable workflow will need to be carefully designed that acquires labeled samples from specific points such as during ventricular assist device installation or cardiac transplant. As instrument sensitivity increases, deducing mitochondrial protein turnover rates from leakage proteins found in plasma or other biofluids may be a potential, though challenging, alternative.

7. Future outlook

Some ongoing developments in protein turnover measurements include the differentiation of protein synthesis and degradation rates, as well as the integration of protein expression and dynamics for systems level analyses. Under constant homeostasis where a steady-state protein pool is maintained, the synthesis rate and the degradation rate of a protein are identical over time. Thus pulse and chase experiments that measure the enrichment or depletion of heavy isotopes are formally equivalent and should give identical values. This assumption does not hold when the proteome shifts towards another steady state, such as when two physiological states are compared (e.g., disease development or altered environment), which compels distinction between whether it is the change in protein synthesis or degradation that drives protein dynamics. Synthesis rates and degradation rates may be distinguished in experiments where changes in the amount of new proteins created and the amount of old proteins removed are separately quantified (as opposed to only ratio of new proteins from the overall protein pool). This may be achieved in vitro as demonstrated using a triple-label SILAC approach [70,71] (with light, medium, and heavy peptides), where the increased in heavy labeled peptides and the decrease in medium labeled peptides can be independently quantified against the light standard. Such methods may be feasibly extended to animal studies using SILAC labels. Alternatively, other approaches that allow the relative changes of the protein pool and the relative change of isotope composition to be simultaneously quantified may be employed, such as using post-extraction chemical labeling (e.g., iTRAQ) [24], label free quantification [40], or SILAM- $^2\text{H}_2\text{O}$ double labeling [45]. Such data allow the relative contributions of synthesis to be calculated from the absolute turnover and changes in protein abundance. When further compared to transcript expression, it will be possible to infer from protein abundance and turnover which genes are regulated at transcriptional, post-transcriptional, or post-translational levels, and to identify genes with impaired proteolysis. Modeling from in vitro studies have suggested that protein synthesis may be more dynamically regulated than protein degradation [23,71], albeit not

without important exceptions that suggest important protein regulations via degradation pathways [71].

To conclude, protein turnover investigations have been buoyed by the advances in proteomics and bioinformatics technologies, and can now reveal the turnover of hundreds of mitochondrial proteins in a single experiment. The resulting protein dynamics data hold the promise for advancing our understanding on mechanistic details of mitochondrial dynamics. For instance, measurements of protein turnover under perturbation will shed light on the proteolytic mechanisms of candidate disease proteins and suggest potential therapeutic strategies to correct their homeostasis. The ability to measure altering protein half-life will allow new hypotheses to be tested. For instance, current investigations on protein degradation pathways rely heavily on pharmacological inhibition of proteases to engender steady-state protein accumulation. Such methods prove limiting in many scenarios such as in defining the repertoire of physiological substrates of the mitochondrial Lon protease, when protein accumulation is not apparent [72]. Finally, one can envision the integration of protein turnover data with other systems-level parameters to enable an integrative description of mitochondrial physiology from molecule to phenotype.

Acknowledgments

This work was supported by the National Institutes of Health awards HL-R37-63901 and HHSN268201000035C, the T.C. Laubisch endowment at UCLA to Peipei Ping; and the American Heart Association fellowships 12PRE11610024 to Edward Lau.

References

1. Pratt JM, Petty J, Riba-Garcia I, Robertson DHL, Gaskell SJ, Oliver SG, et al. Dynamics of protein turnover, a missing dimension in proteomics. *Mol Cell Proteomics*. 2002; 1:579–91.10.1074/mcp.M200046-MCP200 [PubMed: 12376573]
2. Hammerling BC, Gustafsson ÅB. Mitochondrial Quality Control in the Myocardium: Cooperation between Protein Degradation and Mitophagy. *J Mol Cell Cardiol*. 2014; 75:122–30.10.1016/j.yjmcc.2014.07.013 [PubMed: 25086292]
3. Rosca MG, Hoppel CL. Mitochondria in heart failure. *Cardiovasc Res*. 2010; 88:40–50.10.1093/cvr/cvq240 [PubMed: 20668004]
4. Simoni RD, Hill CP, Vaughan M. The Use of Isotope Tracers to Study Intermediary Metabolism: Rudolf Schoenheimer. *J Biol Chem*. 2002; 277:e31.
5. Kresge N, Simoni RD, Hill RL. David Rittenberg: Exploring Porphyrin Synthesis with Duck Blood and Isotope Tracers. *J Biol Chem*. 2005; 280:e12.
6. Shemin D, Bentley R. David Rittenberg. *Biogr Mem Natl Acad Sci*. 2001; 80:257–74. [PubMed: 15202471]
7. Kennedy EP. Hitler's gift and the era of biosynthesis. *J Biol Chem*. 2001; 276:42619–31.10.1074/jbc.R100051200 [PubMed: 11559714]
8. Schoenheimer R, Ratner S, Rittenberg D. STUDIES IN PROTEIN METABOLISM: X. THE METABOLIC ACTIVITY OF BODY PROTEINS INVESTIGATED WITH 1 (–)-LEUCINE CONTAINING TWO ISOTOPES. *J Biol Chem*. 1939; 130:703–32.
9. Rittenberg D, San Pietro A. A STUDY OF THE RATE OF PROTEIN SYNTHESIS IN HUMANS: II. MEASUREMENT OF THE METABOLIC POOL AND THE RATE OF PROTEIN SYNTHESIS. *J Biol Chem*. 1953; 201:457–73. [PubMed: 13044815]
10. Glass RD, Doyle D. On the measurement of protein turnover in animal cells. *J Biol Chem*. 1972; 247:5234–42. [PubMed: 4626918]

11. Rabinowitz M, Zak R. Mitochondria and cardiac hypertrophy. *Circ Res.* 1975; 36:367–76. [PubMed: 163150]
12. Getz GS. Murray Rabinowitz. *Biogr Mem Natl Acad Sci.* 2013
13. Rabinowitz M. Protein synthesis and turnover in normal and hypertrophied heart. *Am J Cardiol.* 1973; 31:202–10. [PubMed: 4265519]
14. Albin R, Dowell RT, Zak R, Rabinowitz M. Synthesis and degradation of mitochondrial components in hypertrophied rat heart. *Biochem J.* 1973; 136:629–37. [PubMed: 4360715]
15. Lipsky NG, Pedersen PL. Mitochondrial turnover in animal cells. Half-lives of mitochondria and mitochondrial subfractions of rat liver based on [¹⁴C]bicarbonate incorporation. *J Biol Chem.* 1981; 256:8652–7. [PubMed: 7263675]
16. Brunner G, Neupert W. Turnover of outer and inner membrane proteins of rat liver mitochondria. *FEBS Lett.* 1968; 1:153–5. [PubMed: 11945279]
17. Fletcher MJ, Sanadi DR. Turnover of rat-liver mitochondria. *Biochim Biophys Acta.* 1961; 51:356–60.10.1016/0006-3002(61)90177-9 [PubMed: 13893581]
18. Martin AF. Turnover of cardiac troponin subunits. Kinetic evidence for a precursor pool of troponin-I. *J Biol Chem.* 1981; 256:964–8. [PubMed: 7451483]
19. Simpson DG, Sharp WW, Borg TK, Price RL, Terracio L, Samarel AM. Mechanical regulation of cardiac myocyte protein turnover and myofibrillar structure. *Am J Physiol.* 1996; 270:C1075–87. [PubMed: 8928735]
20. Khmelinskii A, Keller PJ, Bartosik A, Meurer M, Barry JD, Mardin BR, et al. Tandem fluorescent protein timers for in vivo analysis of protein dynamics. *Nat Biotechnol.* 2012; 30:708–14.10.1038/nbt.2281 [PubMed: 22729030]
21. Yen H-CS, Xu Q, Chou DM, Zhao Z, Elledge SJ. Global protein stability profiling in mammalian cells. *Science.* 2008; 322:918–23.10.1126/science.1160489 [PubMed: 18988847]
22. Andersen JS, Lam YW, Leung AK, Ong SE, Lyon CE, Lamond AI, et al. Nucleolar proteome dynamics. *Nature.* 2005; 433:77–83.10.1038/nature03207 [PubMed: 15635413]
23. Schwanhäusser B, Busse D, Li N, Dittmar G, Schuchhardt J, Wolf J, et al. Global quantification of mammalian gene expression control. *Nature.* 2011; 473:337–42.10.1038/nature10098 [PubMed: 21593866]
24. Jayapal KP, Sui S, Philp RJ, Kok YJ, Yap MGS, Griffin TJ, et al. Multitagging proteomic strategy to estimate protein turnover rates in dynamic systems. *J Proteome Res.* 2010; 9:2087–97.10.1021/pr9007738 [PubMed: 20184388]
25. Maier T, Schmidt A, Güell M, Kühner S, Gavin A-C, Aebersold R, et al. Quantification of mRNA and protein and integration with protein turnover in a bacterium. *Mol Syst Biol.* 2011; 7:511.10.1038/msb.2011.38 [PubMed: 21772259]
26. Doherty MK, Hammond DE, Clague MJ, Gaskell SJ, Beynon RJ. Turnover of the human proteome: determination of protein intracellular stability by dynamic SILAC. *J Proteome Res.* 2009; 8:104–12.10.1021/pr800641v [PubMed: 18954100]
27. Cambridge SB, Gnad F, Nguyen C, Bernejo JL, Krüger M, Mann M. Systems-wide proteomic analysis in mammalian cells reveals conserved, functional protein turnover. *J Proteome Res.* 2011; 10:5275–84.10.1021/pr101183k [PubMed: 22050367]
28. Schwanhäusser B, Gossen M, Dittmar G, Selbach M. Global analysis of cellular protein translation by pulsed SILAC. *Proteomics.* 2009; 9:205–9.10.1002/pmic.200800275 [PubMed: 19053139]
29. Doherty MK, Whitehead C, McCormack H, Gaskell SJ, Beynon RJ. Proteome dynamics in complex organisms: Using stable isotopes to monitor individual protein turnover rates. *Proteomics.* 2005; 5:522–33.10.1002/pmic.200400959 [PubMed: 15627957]
30. Claydon A, Thom M, Hurst J, Beynon RJ. Protein turnover: measurement of proteome dynamics by whole animal metabolic labelling with stable isotope labelled amino acids. *Proteomics.* 2012 In press.
31. Hsieh EJ, Shulman NJ, Dai DF, Vincow ES, Karunadharma PP, Pallanck L, et al. Topograph, a software platform for precursor enrichment corrected global protein turnover measurements. *Mol Cell Proteomics.* 2012; 11:1468–74.10.1074/mcp.O112.017699 [PubMed: 22865922]

32. Westman-Brinkmalm A, Abramsson A, Pannee J, Gang C, Gustavsson MK, von Otter M, et al. SILAC zebrafish for quantitative analysis of protein turnover and tissue regeneration. *J Proteomics*. 2011; 75:425–34.10.1016/j.jprot.2011.08.008 [PubMed: 21890006]
33. Doherty MK, Brownridge P, Owen MAG, Davies SJ, Young IS, Whitfield PD. A proteomics strategy for determining the synthesis and degradation rates of individual proteins in fish. *J Proteomics*. 2012; 75:4471–7.10.1016/j.jprot.2012.03.025 [PubMed: 22484057]
34. Zhang Y, Reckow S, Webhofer C, Boehme M, Gormanns P, Egge-Jacobsen WM, et al. Proteome scale turnover analysis in live animals using stable isotope metabolic labeling. *Anal Chem*. 2011; 83:1665–72.10.1021/ac102755n [PubMed: 21314131]
35. Price JC, Guan S, Burlingame A, Prusiner SB, Ghaemmaghami S. Analysis of proteome dynamics in the mouse brain. *Proc Natl Acad Sci U S A*. 2010; 107:14508–13.10.1073/pnas.1006551107 [PubMed: 20699386]
36. Vogt JA, Hunzinger C, Schroer K, Hölzer K, Bauer A, Schratzenholz A, et al. Determination of Fractional Synthesis Rates of Mouse Hepatic Proteins via Metabolic ¹³C-Labeling, MALDI-TOF MS and Analysis of Relative Isotopologue Abundances Using Average Masses. *Anal Chem*. 2005; 77:2034–42.10.1021/ac048722m [PubMed: 15801735]
37. Guan S, Price JC, Prusiner SB, Ghaemmaghami S, Burlingame AL. A Data Processing Pipeline for Mammalian Proteome Dynamics Studies Using Stable Isotope Metabolic Labeling. *Mol Cell Proteomics*. 2011; 10:M111.010728.10.1074/mcp.M111.010728 [PubMed: 21937731]
38. Lyon D, Castillejo MA, Staudinger C, Weckwerth W, Wienkoop S, Egelhofer V. Automated protein turnover calculations from ¹⁵N partial metabolic labeling lc/ms shotgun proteomics data. *PLoS One*. 2014;9.10.1371/journal.pone.0094692
39. Rosca MG, Vazquez EJ, Kerner J, Parland W, Chandler MP, Stanley W, et al. Cardiac mitochondria in heart failure: decrease in respirasomes and oxidative phosphorylation. *Cardiovasc Res*. 2008; 80:30–9.10.1093/cvr/cvn184 [PubMed: 18710878]
40. Lam MP, Wang D, Lau E, Liem D, Kim AK, Ng DC, et al. Protein kinetic signatures of the remodeling heart following isoproterenol stimulation. *J Clin Invest*. 2014; 124:1734–44.10.1172/JCI73787 [PubMed: 24614109]
41. Price JC, Holmes WE, Li KW, Floreani NA, Neese RA, Turner SM, et al. Measurement of human plasma proteome dynamics with (2)H(2)O and liquid chromatography tandem mass spectrometry. *Anal Biochem*. 2012; 420:73–83.10.1016/j.ab.2011.09.007 [PubMed: 21964502]
42. Busch R, Kim YK, Neese RA, Schade-Serin V, Collins M, Awada M, et al. Measurement of protein turnover rates by heavy water labeling of nonessential amino acids. *Biochim Biophys Acta*. 2006; 1760:730–44.10.1016/j.bbagen.2005.12.023 [PubMed: 16567052]
43. Kasumov T, Ilchenko S, Li L, Rachdaoui N, Sadygov RG, Willard B, et al. Measuring protein synthesis using metabolic (2)H labeling, high-resolution mass spectrometry, and an algorithm. *Anal Biochem*. 2011; 412:47–55.10.1016/j.ab.2011.01.021 [PubMed: 21256107]
44. Kim TY, Wang D, Kim AK, Lau E, Lin AJ, Liem DA, et al. Metabolic labeling reveals proteome dynamics of mouse mitochondria. *Mol Cell Proteomics*. 2012; 11:1586–94.10.1074/mcp.M112.021162 [PubMed: 22915825]
45. Price JC, Khambatta CF, Li KW, Bruss MD, Shankaran M, Dalidd M, et al. The effect of long term calorie restriction on in vivo hepatic proteostasis: a novel combination of dynamic and quantitative proteomics. *Mol Cell Proteomics*. 2012; 11:1801–14.10.1074/mcp.M112.021204 [PubMed: 22984287]
46. Shekar KC, Li L, Dabkowski ER, Xu W, Ribeiro RF, Hecker PA, et al. Cardiac mitochondrial proteome dynamics with heavy water reveals stable rate of mitochondrial protein synthesis in heart failure despite decline in mitochondrial oxidative capacity. *J Mol Cell Cardiol*. 2014; 75:88–97.10.1016/j.yjmcc.2014.06.014 [PubMed: 24995939]
47. Kasumov T, Dabkowski ER, Shekar KC, Li L, Ribeiro RF Jr, Walsh K, et al. Assessment of Cardiac Proteome Dynamics with Heavy Water: Slower Protein Synthesis Rates in Interfibrillar than Subsarcolemmal Mitochondria. *Am J Physiol Hear Circ Physiol*. 2013;10.1152/ajpheart.00933.2012

48. Wang D, Liem DA, Lau E, Ng DC, Bleakley BJ, Cadeiras M, et al. Characterization of human plasma proteome dynamics using deuterium oxide. *Proteomics Clin Appl*. 2014;10.1002/prca.201400038
49. Borek E, Ponticorvo L, Rittenberg D. Protein Turnover in Micro-Organisms. *Proc Natl Acad Sci U S A*. 1958; 44:369–74. [PubMed: 16590208]
50. Rachdaoui N, Austin L, Kramer E, Previs MJ, Anderson VE, Kasumov T, et al. Measuring proteome dynamics in vivo: as easy as adding water? *Mol Cell Proteomics*. 2009; 8:2653–63.10.1074/mcp.M900026-MCP200 [PubMed: 19724074]
51. Claydon AJ, Beynon R. Proteome dynamics: revisiting turnover with a global perspective. *Mol Cell Proteomics*. 2012; 11:1551–65.10.1074/mcp.O112.022186 [PubMed: 23125033]
52. Kalderon B, Gopher A, Lapidot A. Metabolic pathways leading to liver glycogen repletion in vivo, studied by GC-MS and NMR. *FEBS Lett*. 1986; 204:29–32.10.1016/0014-5793(86)81381-3 [PubMed: 3743761]
53. Hellerstein MK, Neese RA. Mass isotopomer distribution analysis at eight years: theoretical, analytic, and experimental considerations. *Am J Physiol*. 1999; 276:E1146–E1170. [PubMed: 10362629]
54. Papageorgopoulos C, Caldwell K, Shackleton C, Schweingrubber H, Hellerstein MK. Measuring protein synthesis by mass isotopomer distribution analysis (MIDA). *Anal Biochem*. 1999; 267:1–16.10.1006/abio.1998.2958 [PubMed: 9918649]
55. Li L, Willard B, Rachdaoui N, Kirwan JP, Sadygov RG, Stanley WC, et al. Plasma proteome dynamics: analysis of lipoproteins and acute phase response proteins with 2H₂O metabolic labeling. *Mol Cell Proteomics*. 2012; 11:M111 014209.10.1074/mcp.M111.014209
56. Guan S, Price JC, Ghaemmaghami S, Prusiner SB, Burlingame AL. Compartment modeling for mammalian protein turnover studies by stable isotope metabolic labeling. *Anal Chem*. 2012; 84:4014–21.10.1021/ac203330z [PubMed: 22444387]
57. Xu T, Venable JD, Park SK, Cociorva D, Lu B, Liao L, et al. ProLuCID, a fast and sensitive tandem mass spectra-based protein identification program. *Mol Cell Proteomics*. 2006; 5:S174.
58. Zong N, Li H, Lam MP, Jimenez RC, Kim CS, Deng N, et al. Integration of Cardiac Proteome Biology and Medicine by a Specialized Knowledgebase. *Circ Res*. 2013; 113:1043–53.10.1161/CIRCRESAHA.113.301151 [PubMed: 23965338]
59. Rogers S, Wells R, Rechsteiner M. Amino acid sequences common to rapidly degraded proteins: the PEST hypothesis. *Science* (80-). 1986; 234:364–8.
60. Tompa P, Prilusky J, Silman I, Sussman JL. Structural disorder serves as a weak signal for intracellular protein degradation. *Proteins Struct Funct Genet*. 2008; 71:903–9.10.1002/prot.21773 [PubMed: 18004785]
61. Dai DF, Karunadharma PP, Chiao YA, Basisty N, Crispin D, Hsieh EJ, et al. Altered proteome turnover and remodeling by short-term caloric restriction or rapamycin rejuvenate the aging heart. *Aging Cell*. 2014; 13:529–39.10.1111/accel.12203 [PubMed: 24612461]
62. Anand R, Langer T, Baker MJ. Proteolytic control of mitochondrial function and morphogenesis. *Biochim Biophys Acta*. 2013; 1833:195–204.10.1016/j.bbamcr.2012.06.025 [PubMed: 22749882]
63. McLelland GL, Soubannier V, Chen CX, McBride HM, Fon EA. Parkin and PINK1 function in a vesicular trafficking pathway regulating mitochondrial quality control. *EMBO J*. 2014; 33:282–95.10.1002/embj.201385902 [PubMed: 24446486]
64. Miwa S, Lawless C, von Zglinicki T. Mitochondrial turnover in liver is fast in vivo and is accelerated by dietary restriction: application of a simple dynamic model. *Aging Cell*. 2008; 7:920–3.10.1111/j.1474-9726.2008.00426.x [PubMed: 18691181]
65. Miller BF, Robinson MM, Bruss MD, Hellerstein M, Hamilton KL. A comprehensive assessment of mitochondrial protein synthesis and cellular proliferation with age and caloric restriction. *Aging Cell*. 2012; 11:150–61.10.1111/j.1474-9726.2011.00769.x [PubMed: 22081942]
66. Yuan CL, Sharma N, Gilge DA, Stanley WC, Li Y, Hatzoglou M, et al. Preserved protein synthesis in the heart in response to acute fasting and chronic food restriction despite reductions in liver and skeletal muscle. *Am J Physiol Endocrinol Metab*. 2008; 295:E216–22.10.1152/ajpendo.00545.2007 [PubMed: 18445754]

67. Emson CL, Fitzmaurice S, Lindwall G, Li KW, Hellerstein MK, Maibach HI, et al. A pilot study demonstrating a non-invasive method for the measurement of protein turnover in skin disorders: application to psoriasis. *Clin Transl Med.* 2013; 2:12.10.1186/2001-1326-2-12 [PubMed: 23773261]
68. Fanara P, Wong PY, Husted KH, Liu S, Liu VM, Kohlstaedt LA, et al. Cerebrospinal fluid-based kinetic biomarkers of axonal transport in monitoring neurodegeneration. *J Clin Invest.* 2012; 122:3159–69.10.1172/JCI64575 [PubMed: 22922254]
69. Busch R, Neese RA, Awada M, Hayes GM, Hellerstein MK. Measurement of cell proliferation by heavy water labeling. *Nat Protoc.* 2007; 2:3045–57.10.1038/nprot.2007.420 [PubMed: 18079703]
70. Boisvert F-M, Ahmad Y, Gierlinski M, Charriere F, Lamont D, Scott M, et al. A Quantitative Spatial Proteomics Analysis of Proteome Turnover in Human Cells. *Mol Cell Proteomics.* 2012; 11:M111.011429–M111.011429.10.1074/mcp.M111.011429
71. Kristensen AR, Gsponer J, Foster LJ. Protein synthesis rate is the predominant regulator of protein expression during differentiation. *Mol Syst Biol.* 2013; 9:689.10.1038/msb.2013.47 [PubMed: 24045637]
72. Lau E, Wang D, Zhang J, Yu H, Lam MP, Liang X, et al. Substrate- and isoform-specific proteome stability in normal and stressed cardiac mitochondria. *Circ Res.* 2012; 110:1174–8.10.1161/CIRCRESAHA.112.268359 [PubMed: 22456183]

Highlights

- Protein turnover is an important component of mitochondrial quality control.
- Proteomics techniques now allow protein turnover to be measured on a large scale.
- Individual mitochondrial proteins exhibit very different turnover rates in vivo.
- Protein turnover may alter independently from protein expression during disease.

Figure 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): (Lower panel): incorporation rate of stable isotope labels correspond 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 $^2\text{H}_2\text{O}$ results in a shift in the fractional abundance of isotopomers in the envelope.

Figure 2. Heterogeneity of mitochondrial protein turnover in multiple tissues

The scatterplots show the standardized log turnover rates (Z score) of 444 mitochondrial proteins commonly measured in mouse heart, liver, and kidney. Comparisons between **A.** heart and liver; and **B.** liver and kidney; are shown. Each data point represents a distinct protein. The red diagonal line represents 1:1 in standardized turnover rates in two respective tissues, whereas dashed lines represent the positions of data points where a protein turns over markedly faster or slower in one tissue (+1.5 or -1.5 standard deviations from average). Proteins that differ markedly in relative turnover rates between two tissues are marked, e.g., a cluster of mitochondrial ribosomal proteins (MRPs) can be seen that turn over relatively faster in the heart than in the liver. Two inner membrane carriers for amino acids/amino acid precursors (SLC25A29 and SLC25A26) turn over relatively faster in the liver than in the kidney. These differences are an indication of the tissue specificity of mitochondrial biology and the accompanying heterogeneity in the regulation of protein turnover rates.

Table 1
Meta-analysis of mitochondrial turnover rates measured in multiple mouse tissues

The turnover rates of mitochondrial proteins from multiple tissues reported in a number of recent whole-animal labeling experiments are shown.

Tissue	Turnover rates (d ⁻¹)	Average	Isotope label	Mouse strain	N° mito proteins*	Reference
Blood	0.210 [0.090 – 0.326]	0.210	¹⁵ N	FVB	22	[35]
	0.149 [0.095 – 0.566]		² H ₂ O	Hsd:ICR	545	[40]
Liver	0.167 [0.113 – 0.673]	0.155	¹⁵ N	FVB	278	[35]
	0.150 [0.100 – 0.380]		² H ₂ O	C57BL/6J	91	[45]
	0.144 [0.093 – 0.477]		² H ₂ O	Hsd:ICR	635	N/A#
Kidney	0.147 [0.070 – 0.306]	0.146	[² H ₆]-valine	C57xBALBc	10	[30]
	0.045 [0.021 – 0.198]		² H ₂ O	Hsd:ICR	537	[40]
Heart	0.039 [0.014 – 0.117]	0.044	[² H ₆]-valine	C57xBALBc	17	[30]
	0.048 [0.024 – 0.285]		[² H ₃]-leucine	C57BL/6J	258	[61]
	0.034 [0.024 – 0.117]	0.034	[² H ₆]-valine	C57xBALBc	3	[30]
Skeletal Muscle	0.027 [0.013 – 0.140]		¹⁵ N	FVB	153	[35]
Brain	0.011 [0.007 – 0.105]	0.019	¹⁵ N	DBA/2J	17	[34]

Turnover rates are reported as median and [5th to 95th percentile], when available.

* To allow for standard comparison, mitochondrial proteins are here defined as the 1,008 proteins with the “mitochondrial” keyword annotation at SwissProt (release 2014_07, taxonomy: 10090, keyword: KW-0496; reviewed). The table includes studies that specifically enriched for mitochondrial proteins and those that did not.

Unpublished kidney data were from the same dataset as described in Lam et al. [40].