Published in final edited form as:

Expert Rev Proteomics. 2015 April ; 12(2): 133–146. doi:10.1586/14789450.2015.1024227.

Spatial and temporal dynamics of the cardiac mitochondrial proteome

Edward Lau^{1,*}, Derrick Huang¹, Quan Cao¹, T. Umut Dincer¹, Caitie M. Black¹, Amanda J. Lin¹, Jessica M. Lee¹, Ding Wang¹, David A. Liem¹, Maggie P.Y. Lam¹, and Peipei Ping^{1,2,*}

¹Departments of Physiology, The NHLBI Proteomics Center at UCLA¹, UCLA David Geffen School of Medicine, Los Angeles, CA 90095, USA

²Departments of Medicine, and Bioinformatics², NIH Center of Excellence in Big Data Computing at UCLA², UCLA David Geffen School of Medicine, Los Angeles, CA 90095, USA

Summary

Mitochondrial proteins alter in their composition and quantity drastically through time and space in correspondence to changing energy demands and cellular signaling events. The integrity and permutations of this dynamism are increasingly recognized to impact the functions of the cardiac proteome in health and disease. This article provides an overview on recent advances in defining the spatial and temporal dynamics of mitochondrial proteins in the heart. Proteomics techniques to characterize dynamics on a proteome scale are reviewed and the physiological consequences of altered mitochondrial protein dynamics are discussed. Lastly, we offer our perspectives on the unmet challenges in translating mitochondrial dynamics markers to the clinic.

Keywords

Mitochondria; protein dynamics; cardiovascular disease; spatial dynamics; temporal dynamics

1. Introduction

The mammalian heart is about one-third mitochondria by volume, and requires a staggering 6 to 30 kg of ATP per day to maintain circulation (1, 2). At least two mitochondrial subpopulations reside in the myocardium – the interfibrillar mitochondria which embed within myofibrils, and the subsarcolemmal mitochondria which abut the cellular membrane – to support its contractile and electrophysiological energy expenditure. A common observation in cardiac diseases is the diminishment of mitochondrial energetic output and the activation of mitochondrion-mediated cell death pathways (3, 4). Consequently,

*Corresponding authors: Edward Lau (edwardlau@ucla.edu), Peipei Ping (peipeiping@earthlink.net), 675 Charles E Young Drive S, MRL 1-619, University of California at Los Angeles, Los Angeles, CA 90095, USA, +1 (310) 267-5624.

Financial & competing interests disclosure

This work was supported in part by a NHLBI Proteomics Center Award HHSN268201000035C and NIH Awards R37HL063901 and R01HL123060, and the UCLA Laubisch Endowment to P Ping. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

significant efforts have been devoted to understand this organelle and the protein constituents responsible for its metabolic and signaling functions. The mitochondrial proteome contains a specialized composition of proteins, which vary qualitatively and quantitatively amongst mitochondria of different species and cell types (5, 6). Advances in quantitative proteomics have in the past decade revolutionized our understanding of the cardiac mitochondrial proteome, beginning with the elucidation of the identity of various mitochondrial proteins, leading to sophisticated, quantitative surveys of protein expression levels that are becoming a fount of understanding on disease mechanisms in general.

A more recent realization in proteomics studies is that disease discriminants may be found not only in the alterations of the abundance of mitochondrial proteins, but also through their dynamic behaviors over space and time. Protein dynamics may be described on multiple levels. This review will be confined to the transient location and translocation of proteins in and out of the mitochondrial membranes upon defined stimuli, and the stability and degradation of mitochondrial proteins within and without the mitochondrion. Mitochondria themselves are highly mobile and dynamic entities that continually change in relation to the cell state at the whole-organelle level. Vigorous research in cardiovascular and other branches of biomedical sciences has sprouted to examine their biogenesis, movement, morphology, fission, fusion, and autophagic destruction, each of which has been observed to partake in the progression towards disease. The reader is referred to several excellent reviews of such organelle-level dynamics in the heart (3, 7–9), but the topic will not otherwise be discussed here except insofar as to note that organelle-level dynamics can enable inter-mitochondrial spatial dynamics of certain mitochondrial proteins. For instance, whilst two individual mitochondrial units liaise, respiratory complex subunits may traverse spatially from one to the other, as may certain mitochondrial matrix proteins (10, 11). This phenomenon has been theorized to function in the assurance of minimal acceptable quality of mitochondria in the cell and to rescue subsets of individual organelles under duress via the apportionment of undamaged proteins (12), possibly through an as-yet undefined sorting mechanism that would be necessary for such endergonic processes to proceed. Nevertheless, as of the moment the protein dynamics of inter-mitochondrial exchange remains virtually unknown, in part because of the short timescale (seconds) involved in such contact.

2. Spatial dynamics of mitochondrial proteins

2.1 Defining the mitochondrial proteome

The spatial distribution of a protein – to which parts of the cell it localizes and with which proteins it interacts – dictates its functional specificity. This property is routinely harnessed to understand signaling mechanism and responses to changing cellular conditions in single-protein inquiries. At the global level, spatial distribution can be used to inform on the potential functions of unknown proteins. The definition of the mitochondrial proteome inherently contains spatial information, in that proteins are either localized within or without the lipid boundary of mitochondrial membranes. Hence the simplest instance of spatial dynamics study is to ask the question of what proteins are localized to the mitochondria at a particular cell state. Non-proteomics approaches such as immunofluorescence microscopy are widely used to examine the subcellular localization of relatively few targeted proteins,

but investigations of small subsets of proteins often lead to a limited if not distorted view of the complex regulatory networks of biology, and can even lead to false discovery by obscuring the posterior probability of particular events within the system. Hence for applications where a large number of proteins are concerned, high-throughput methods capable of characterizing protein localization at the global level are typically sought. Pre-sequence analysis and co-expression analysis have been used to deduce organelle localization, but in general where the protein is localized at an organelle resolution or whether it is indeed expressed at a particular cell type in a particular physiological state cannot be accurately predicted from nucleotide sequences alone, and thus this is a question under the purview of experimental proteomics.

Proteomics experiments, coupled with targeted mitochondrial isolation via ultracentrifugation, have been very successful in delineating the content of the mitochondrial proteome in multiple organs. In fact, the elucidation of the mitochondrial sub-proteome (as was that of other organelles like ER and chloroplast) in different tissues has been a principal experimental objective of the discipline of proteomics for many years. The history and methods of mitochondrial protein identification have been reviewed in (13), to which the reader is referred for a more thorough discussion. Mitochondrial proteomics studies have been made possible by elaborate fractionation protocols that were devised to isolate mitochondrial membranes such that they could be analyzed by mass spectrometry in a targeted manner. With organelle isolation, the purity of the preparation was a primary concern, as they define the input to proteomics experiments, and contaminations from other cellular compartment would obviously lead to spurious detection and wrongful assignment of a non-mitochondrial protein as mitochondrial. Isolation techniques commonly make use of custom differential centrifugation over a density gradient, which separates mitochondria from other membrane-bound cellular compartments based on their buoyant density. For cardiac tissues, mitochondrion isolation techniques are relatively mature, and various modifications have also been devised to promote organelle viability and to prevent leakage and rupture, which may alter protein abundance. Cardiac mitochondrial preparations are generally free of lysosomes and other intracellular membranes and yield pure entities as seen under electron microscopy, though individual protocols need to be optimized for different tissue and cell types that may contain different amounts and morphologies of mitochondria and other intracellular membrane structures. Specialized techniques are also adopted to separate interfibrillar and subsarcolemmal populations, whereas procedures have also been described to solubilize outer membranes to reveal mitoplasts. However, it should be noted that even with stringent multi-step isolation, potential protein contaminants may still remain as common blood and immune proteins such as hemoglobins and complement factors are frequent observations. Turnover analysis suggests that these proteins are likely to come from blood and may have attached to mitochondrial surfaces during isolations.

Proteomics experiments culminated in a rapidly growing catalog of cardiac mitochondrial proteins and their functions in cardiac health and disease. A recent report from our group placed the number of cardiac mitochondrial proteins at approximately 1,500 proteins (6), which may be categorized into several major functional groups (Figure 1). Works from other groups employed mitochondrial proteomics to assess the changing quantity, phosphorylation, and S-nitrosylation of mitochondrial proteins in models of cardioprotection

from ischemic injuries (reviewed in (14, 15)). Mitochondrial proteomics also revealed decreased tricarboxylic acid (TCA) cycle enzymes in cardiac mitochondria in diabetes model that contrasted with their preservation in hepatic, renal, and cerebral mitochondria of the same animals and could explain the origin of diabetic cardiomyopathy. More recently, the remodeling of the mitochondrial proteome was assessed in heart failure (transverse aortic constriction) and catalase-cardioprotection models (16), and proteomics was used to pinpoint the elusive molecular identity of the MitoK_{ATP} channel (17).

2.2. Dual localization of mitochondrial proteins

Despite the many exciting applications of mitochondrial proteomics in the study of heart diseases, we are nowhere close to a universally recognized mitochondrial proteome (cf. the relative concreteness of a bacterial proteome). Certain mitochondrial proteins may form a “core” mitochondrial proteome, whereas others may vary between mitochondrial types (6, 18). Yet other proteins may be facultative in their mitochondrial membership, localizing to the organelle only under specific time and conditions. It should be noted that the quest for an absolutely pure mitochondrial proteome is rooted in the paradigm that a protein ought only to have one primary molecular function and thus one primary localization in the cell. This notion, much like the conceptualization of cellular proteins as a free floating soup of single molecules chancing into every possible interaction partner, is a useful biological shorthand and but perhaps an oversimplification of the architecture of the cell. Hence, whilst stringent purification protocols are necessary to determine the core constituents of the mitochondrial proteome and have led to a sizable corpus of knowledge on mitochondrial physiology, the very act of stringent purification also carries the latent cost of trading off the chance to detect the dynamically- and facultatively- mitochondrial proteins that may act to exchange biological information between two or more cellular locales and thus may play an more important role in cellular physiology than the constitutively mitochondrial proteins. The ability to determine these loosely mitochondrial proteins from noise has in part been propelled by unbiased enrichment approaches that aim to make use of continuous density gradients to enrich rather than completely purify individual organelles, followed by statistical analysis to cluster individual proteins and classify their probable organelle membership.

Given that contamination is both an experimental challenge as well as a biological reality, techniques include the Localization of Organelle Proteins by Isotope Tagging (LOPIT) (19, 20), Protein Correlation Profiling (PCP) (18, 21), and similar methods have been instrumental in providing a comprehensive view of protein localization, dual localization, and translocalization (Figure 2). Much like targeted organelle purification, multi-compartment enrichment techniques rely on differential sedimentation in a density gradient centrifugation to spatially separate cellular parts into multiple fractions, which correlates with known organellar compartments and can be individually collected for protein identification and quantification via high-resolution mass spectrometry (19, 20, 22). A major distinction of multi-compartment enrichment techniques such as LOPIT and PCP is the assumption that proteins from subcellular compartments will co-fractionate and subsequently exhibit similar gradient distributions that can be identified as clusters, even if no single layer on the density gradient would contain only a single purified organelle. While

LOPIT employs isotopically coded tags (e.g., iCAT, iTRAQ) for quantification, PCP utilizes label-free techniques to determine distribution profiles. Multivariate statistical analyses and machine learning methods including principal components analysis and partial least squares discriminant analysis models are then applied to correlate gradient profiles of the fractions with known organelle markers to assign the unknown primary localization of an investigated protein in a particular cell state (20). Despite the fact that these techniques necessarily rely on the existence of previously established organelle markers, an accumulating number of suitable fraction anchors are now known and LOPIT/PCP have been successfully employed to localize proteins in a growing number of subcellular compartments including mitochondria, endoplasmic reticulum and Golgi, lysosome, and plasma membrane (19, 20, 22). A primary advantage of continuous gradient techniques over more targeted organelle isolation methods is that robust statistical methods can be employed on multivariate data to distinguish authentic protein localizations from contaminants or separation noise. They also provide high-throughput localization assays for multiple cellular compartments. Most importantly, multivariate data analysis techniques have the potential to characterize the dynamic and coordinated changes of protein localization at the proteome level (20). By providing data on multiple organelles simultaneously, multivariate protein fraction data can be modeled to circumvent off-target organelle cross-contamination and provide improved statistics on differentiating protein translocation from noise. The singularity of a translocation event, e.g., that of a mitochondrial protein traveling to the nucleus, can be verified and the possible contributions of artifactual contamination rejected by contrasting the protein to the changes in cellular distribution of the totality of mitochondrial proteins. Coupled to multivariate data analysis, these techniques can also be utilized to monitor how numerous proteins dynamically move between multiple subcellular localizations in a temporal- and condition- dependent manner (21, 23).

These and other organellar proteomics experiments generally corroborate the fluidity of the boundary between mitochondrial and non-mitochondrial proteins. The dual localization of mitochondrial proteins can be easily observed experimentally in both the identification of proteins conventionally seen as extra-mitochondrial (e.g., histones) in purified mitochondrial preparations, and the identification of classically mitochondrial proteins (e.g., respiratory chain subunits) in mitochondrion-depleted fractions. These may have been conveniently ignored in the past or explained away as artifactual experimental contaminations, but as evidences mount in both large-scale and targeted studies, they can be expected to be gradually accepted as the cellular norm. A study in yeast showed dual localization of about one third of examined mitochondrial proteins (24). Sharing between mitochondrial and nuclear localization is particularly intriguing, given the relatively different densities of the two organelles should render cross-contamination an unlikely explanation of ectopic protein residence. A query on the protein knowledgebase Uniprot returns only 13 human proteins that are annotated as being both in the nucleus and on the mitochondrial inner membrane, including PHB, SRC, TIMM50, AIF, and NDUFAF13. This is almost certain to be an underestimate by some distance. In non-cardiac cells, as many as 1,000 mitochondrial proteins were found to have dual localization between the mitochondrion and the nucleus (25). Up to a hundred mitochondrial proteins in the mammalian heart could be seen in proteomic profiling of highly purified nuclear preparations, including ATP synthase subunits

and TCA cycle proteins (26), whereas highly purified cardiac mitochondrial preparations contain histones and lamin A (6, 27). In non-cardiac fibroblasts, over 300 proteins could be detected to partition in nuclear fractions yet are ordinarily annotated as mitochondrial (23). This phenomenon does not appear to arise from an intrinsic noisiness in spatial targeting, because dual-localized proteins were found to differ significantly in sequence features in their targeting peptides (24). In many instances, the mislocalization of non-mitochondrial protein to the mitochondrion (e.g., amyloid precursor APP, PEX26, etc.) and vice versa (DRP1) are causal pathogenic events in multiple disorders including Alzheimer's disease and Charcot-Marie-Tooth disease (28–31). Furthermore, a mitochondrial AAA+ ATPase (ATAD1) has been found to function to remove ER tail-anchored proteins that are mislocalized to the mitochondrion (32, 33), lending further credence to the view that the observed dual localizations are nonrandom but in accordance to cellular purpose.

It should be noted, however, a caveat in the interpretation of these and other proteomics data is that the differentially localized proteins may in fact be multiple obscured alternative splicing isoforms (34). For example, a splice isoform of paraplegin, a mitochondrial protein, was found to localize instead to the ER in targeted investigation (35). At present, it is often difficult if not impossible to differentiate splice isoforms in a large-scale experiment, because the shotgun nature of proteomics experiments leads to identical peptide fragments from different isoforms. Secondly, many isoforms are unknown either on the transcript or the protein level and are not present in commonly utilized protein sequence databases.

Spatial localization to cellular compartments is dynamic, in that many proteins are known that facultatively translocate to mitochondria based on physiological status or other stimuli but otherwise reside primarily in another locale. This process when generalized to a global scale probably constitutes significant exchange between the mitochondrial proteome and the cellular components. Numerous examples exist from individual-protein studies where proteins translocate into mitochondria following stimulus (e.g., Bim, Bad, Bmf), and only two examples of particular interest to cardiac diseases will be briefly reiterated here as archetypical embodiments. Firstly, STAT3 is a transcription factor which, besides being a nuclear effector of JAK and EGFR pathways, also functions in the mitochondria to regulate respiratory activities and cell death in the heart. Cardiac-specific STAT3 knockout leads to heart failure; translocation of STAT3 into the mitochondria is mediated by GRIM-19, a complex I regulatory protein (36). The introduction of STAT3 into mitochondria may suppress mitochondrial permeability transition, a point of convergence of multiple signaling pathways that precede programmed necrosis (37). In another example, the translocation of cardiac PKCepsilon to the mitochondria is a pivotal event in protecting the heart from cell death from multiple cardioprotective methods (38). Upon activation, PKCepsilon moves from the cytosol to mitochondria where it interacts with Lck, a Src family tyrosine kinase, to form a module which then proceeds to phosphorylate downstream targets in the mitochondria. The disruption of mitochondrial translocation abolishes cardioprotection. Of note, STAT3 and PKCepsilon do not appear to be commonly found in proteomics experiments (e.g., (6, 39)), which may be explained by their transient and inducible translocation into the organelle, which would in turn cause them to be absent in mitochondria when sampled under basal conditions or otherwise to be washed out by stringent purification methods.

In the above cases, inducible translocation into mitochondria rely on adaptor proteins or chaperones, e.g., STAT3 by GRIM-19 (36) and PKCepsilon by Hsp90 (40). This appears to be a general observation, and coordinated import and folding of proteins into the mitochondrion by chaperon is a conserved mechanism of introducing proteins into the organelle (41). However, in certain instances global alterations in protein localization may also be directly attributable to impairments in protein import machineries including translocase of the outer membrane (TOM) and the translocase of the inner membrane (TIM). For instance, decrease in TOM20 upon ischemic injury has been implicated to lead to subsequent changes in mitochondrial proteome makeup, which is alleviated by cardioprotective preconditioning (42). Furthermore, decreases in TOM20 and TIM23 are associated with impaired protein import into mitochondria in diabetic interfibrillar mitochondria in the heart (43), whereas decreases in HSP70 have also been observed to alter mitochondrial protein import in the diseased heart (44).

Proteins may also first locate to mitochondria then retrotranslocate from the organelle under stimulus, including the well-known example of cytochrome c in apoptosis. Upon apoptosis in the ischemia-reperfusion injured heart, cytochrome c exits mitochondria through the oligomeric Bax/Bak pore, which permeabilizes the mitochondria outer membrane. Cytosolic cytochrome c forms apoptosome in the cytosol to activate the caspase cascade. In parallel, the flavoprotein apoptosis-inducing factor (AIF) also exits mitochondria and promote caspase-independent cell death processes, as do Smac/Diablo, and HrtA2/Omi and endonuclease G to participate in apoptosis (45, 46). Proteins can also egress from mitochondria under permeability transition (47) or through escaping mitophagy (48). In non-cell-death scenarios, two examples of retrotranslocation of a mitochondrial protein are given here that are of particular interest to cardiac research. The first is hexokinase II. Hexokinases are the first step of glycolysis, but also regulate cardiac cell death and cardioprotection following ischemia-reperfusion injury (49). HKII is a major isoform expressed in the adult heart, and its activities are regulated by its dynamic localization to cardiac mitochondria. HKII attaches to the outer surface of mitochondria via tethering to VDAC (50, 51), a major mitochondrial outer membrane protein and a regulatory component of the permeability transition pore. Under high-glucose conditions, HKII can dynamically translocate away from mitochondria into the cytosol, a phenomenon that coincides with a switch in its molecular functions from the promotion of catabolic glycolysis to anabolic glycogen synthesis (51). Dynamic mitochondrial localization of hexokinase hence acts as a connection between metabolism and cardiac injury, as detachment of mitochondrial HK is thought to promote permeability transition pore opening and cell death. Another example of dual localization of mitochondrial proteins can be found in nuclear factor-erythroid 2-related factor 2 (NRF2), a pleiotropic transcription factor responsible for the antioxidant response element (ARE) in the heart as a major mechanism of defense against reactive oxygen species (52). Deletion of NRF2 exacerbates cardiac hypertrophy after overload (53). Under basal conditions, NRF2 is likely tethered to the mitochondrial surface through complexing with the mitochondrial outer membrane phosphatase phosphoglycerate mutase 5 PGAM5 via a complex that also contains Kelch-like ECH-associated protein 1 (KEAP1) (54). Under ROS, NRF2 translocates into the nucleus for signaling. Hence, NRF2 may form a part of a retrograde communication from mitochondria back to the nuclear genome.

Whereas initial single-pathway studies have focused on the above proteins individually as a reflection of their idiosyncratic signaling modality, accumulating data now favor their conceptualization as part of a generalizable phenomenon of the proteome, namely that the organellar proteome changes between disease and health not only in terms of the expression of statically mitochondrial proteins, but also in the concerted transformation of spatial distribution across subcellular compartments. The extent of this translocation is not known, but previous investigation by our group has found the occurrence of mitochondrial-targeted proteins accumulating in cytosolic fractions during ischemic injury to be widespread and to include proteins on the inner membrane or the matrix (55). In non-cardiac fibroblasts, particular subsets of mitochondrial-annotated proteins including ATP synthases and fatty acid oxidation proteins were shown by others to translocate out of nucleus following oxidative stress (23).

2.3 Potential mechanisms of translocation

For proteins that translocate away from mitochondria dynamically, a pertinent question is what the mechanisms may be for dynamic translocation and retrotranslocation, especially regarding proteins that cross the otherwise impermeable inner membrane (Figure 3). Besides the Bax/Bak pore and permeability transition pore during cell death, one possibility lies in the ER-assisted degradation (ERAD) components including Vcp/Cdc48/p97, which are also known to associate with mitochondria (56), and are responsible for the extraction and ubiquitin-mediated degradation of some mitochondrial proteins including the outer-membrane mitofusin (56). Certain intra-mitochondrial proteins including uncoupling proteins 1, 2, and 3 (UCP1, UCP2, UCP3) were also shown to require ubiquitination and cytosolic proteasomes for turnover (57–59), but whether intra-mitochondrial proteins may egress from the organelle via identical or similar export mechanisms is an open question. Secondly, recent studies have revealed another potential mechanism for retrotranslocation of proteins from the mitochondrion that may serve to define mitochondrial interactions with other cellular locales such as peroxisomes and lysosomes. These translocations are mediated via retrograde transport of proteins originating from the mitochondria packaged into mitochondrial-derived vesicles (MDVs). MDVs form after the incorporation of specific protein species that may derived from any of the mitochondrial membranes or matrix proteins including TOM20 and PDH, and may play a significant role in cellular homeostasis, waste degradation, and the removal of oxidative damage (60, 61). MDVs may function as a parallel mitochondrial protein quality control measures to mitophagy and individual substrate proteolysis. The lysosome is a primary destination of MDVs and their associated protein cargoes including VDAC and UQCRC2, where significant increases in MDV generation and accumulation have been demonstrated to occur under sub-lethal oxidative stressors and under treatment with lysosomal inhibitors in cultured cells, respectively (60, 61). Increased MDV generation suggests that the pathway may function in the degradation of mitochondrial proteins damaged by oxidative stress. MDVs have also been demonstrated to deliver proteins to the peroxisome. MAPL, a membrane protein ligase that has been shown to facilitate fission, is the only currently known protein to translocate to peroxisomes (60, 61). Disrupted MDV generation may have disease implications, e.g., the protein kinase PINK1 and the cytosolic ubiquitin E3 ligase parkin are both required for MDV-to-lysosome functionality. The primary studied functions of PINK1/parkin in mitochondrial quality

control corroborates that MDV generation may be a stress response mechanism. Further elucidation of MDV-mediated cellular mechanisms will require quantitative and qualitative MS techniques to be developed that can identify the molecular actors involved in MDV generation and transport.

Lastly, spatial dynamics may also involve intra-mitochondrial changes in protein distributions, e.g., differential complex formation. Unfortunately, current knowledge on a dynamic interactome is woefully incomplete, as existing protein-protein interaction databases distinguish only species and have almost no resolution with regard to cell type, cellular compartments, or disease state. Nevertheless, the importance of such finer levels of spatial interactions can be observed in the rare instances where such processes have been investigated in detail, such as in the assembly and disassembly of the large protein complexes and supercomplexes of the respiratory chain. Their biological and therapeutic implications of respiratory chain assembly defects have been well demonstrated in a number of instances where assembly errors can lead to respiratory defects in mitochondria (62–64). Mutations and disorders of specific complex I subunits can disrupt complex formation and functions, leading to diseases including Mitochondrial Encephalomyopathy, Lactic Acidosis and Stroke-like episodes (MELAS) and Leigh Syndrome (65, 66). Mutations affecting the assembly of one complex may also have effects on other complexes, with complex I-specific genes producing deficiencies in both complex I and complex IV (63). Recent investigations have further defined higher level organization into respiratory supercomplex, or respirasome, elucidating interactions and electron pathways among complexes I, III, and IV (64), and examining the sequence and kinetics of assembly that may explain structural interdependency among respiratory complexes to produce a multistep supercomplex assembly model (67). Future proteomics experiments perhaps using unbiased cross-linkers may help elucidate details of spatial dynamics in proteins that are not by default sequestered into well-defined stable complexes.

3. Temporal dynamics of mitochondrial proteins

3.1 Protein turnover in the mitochondria

The functions of the cardiac mitochondrion also depend on the integrity and homeostasis of its protein constituents. The cardiac mitochondrial proteome is a dynamic entity that constantly changes and renews: protein pools renew at characteristic turnover rates, whereas changes in protein half-life in heart disease models suggest that the proteome pools may alter in functions without apparent changes in protein structure or abundance (68–70). The relevance of mitochondrial protein spatiotemporal dynamics to disease mechanisms is therefore an intensively pursued research subject.

Respiratory activities in the mitochondria constitute a significant source of ROS in the cell. Mitochondrial proteins in proximity to ROS are at risk for oxidative damage, which may account for the reason why the mitochondrion possesses its own intricate system of proteases and chaperones (71). Protease systems that exist inside mitochondria include several ATP-dependent protease complexes: the Lon protease homolog LONP1, the ClpXP protease complex, and the m-AAA and i-AAA metalloproteinase complexes, among which LONP1 is the best characterized. The Lon protease is a homoheptameric complex of LONP1

monomers conserved in all domains of life, and contains distinct domains for ATP binding, substrate recognition, and proteolysis. The substrate-recognition domain is thought to bind to exposed hydrophobic domains from potential protein substrates (72), through which LONP1 promotes the degradation of mildly oxidized proteins (73–75) and impedes protein carbonyl accumulation in vitro (76, 77). Circumstantial evidence on the importance of intra-mitochondrial proteases can be found in that fact that mutations in multiple complexes have been associated with severe developmental defects in human and cellular dysfunctions in animal models. Mutations in the gene encoding for the LONP1 protein lead to the cerebro-oculo-dento-auriculo-skeletal (CODAS) syndrome in human with particularly debilitating presentations in the heart and the brain (78). Mutations in the gene encoding for the proteolytic CLPP subunit of the ClpXP protease complex is one of the genetic causes that lead to Perrault syndrome with sensorineural hearing loss and ovarian dysgenesis (79). Finally, mutations in paraplegin, a component of the m-AAA metalloproteinase complex, cause autosomal recessive hereditary spastic paraplegia with respiratory disorders and neurodegeneration (80). In animal models, deletion of the Lon homolog in Yeast (Pim1) leads to premature aging (81). The deleterious effect of mitochondrial protein turnover perturbations has been linked to mtDNA maintenance and accumulation of protein carbonylation.

Notwithstanding the above, and somewhat surprisingly given also the importance of mitophagic turnover on the organelle level, mitochondrial proteins are in fact significantly more stable than proteins in other examined cellular compartments (68, 82). In the mouse heart, mitochondrial proteins are replaced at an average rate of 5% per day, although with large distribution that spans over 10-fold. By contrast, cytosolic proteins in the heart are replaced at significantly higher rates, at ~10% per day (68). Furthermore, although mitochondrial protease complex can account for up to 0.1% of all proteins in the mitochondrial proteome (83), molecular data on the physiological significance of their photolytic activity have not been entirely conclusive (84, 85). Thus far a relatively limited number of mitochondrial proteins have been conclusively demonstrated to be LONP1 substrates in vivo (86, 87), most notable of which is the mitochondrial transcription factor A (TFAM), through the action on which LONP1 may modulate mitochondrial DNA copy number (87). Curiously, genetic ablation of the LONP1 homolog in yeast (Pim1) causes impairments in respiration and mitochondrial functions (88) but results in the accumulation of relatively few proteins in the yeast mitochondrion (less than 10% of 2D gel spots) (89), an observation that is potentially attributable to compensatory effects from decreased protein synthesis of the Pim1 substrates in Pim1 strains. Hence the general substrate repertoire of LONP1 and other mitochondrial proteases remains poorly defined, a situation which limits our understanding on which of their proteolytic substrates, if any, are responsible for the phenotypic outcome when the proteases are suppressed or mutated. Although overall protein expression may be balanced by expression, protein turnover is expected to alter and cause the resulting phenotypic changes. As of the moment, there is not yet direct evidence that alterations in these proteases do impede the rates of protein turnover, which will be an interesting topic of investigation.

3.2 Measuring the temporal dynamics of mitochondrial proteins

Many studies have detailed the turnover rates of mitochondrial proteins in vitro using a variety of techniques, from fluorescence timer to artificial inhibition of protein synthesis. However, our understanding of the temporal dynamics of proteins in the heart was hampered by difficulty to measure physiologically relevant protein half-life in vivo. This veil is slowly being pulled back by recent advances in several techniques to quantify the turnover rates of proteins in living animals, which together are enabling a new dimension of mitochondrial protein dynamics to be characterized. These techniques are primarily driven by the use of stable isotope precursors such as ^{15}N , $^2\text{H}_2\text{O}$, or pre-labeled amino acids, in conjunction with high-resolution mass spectrometry and computational modeling to deduce the kinetic behaviors of isotope incorporation (90–92). Two major considerations to successfully measure mitochondrial protein turnover on a large scale are the accurate deduction of precursor level in the organism in order to calculate turnover rates from precursor-product relationship, and the availability of automated computational software to increase the throughput of data analysis. In recent publications, the turnover rates of up to 500 or more cardiac mitochondrial proteins may be deduced in a single experiment (68, 92, 93). For technical details in the measurement of protein turnover rates, the reader is referred to an excellent recent review by Claydon and Beynon (94) and to a previous review article from our group (95).

Through large-scale turnover measurements it is now known that there is marked heterogeneity in the turnover rates of individual mitochondrial proteins. Individual mitochondrial proteins can vary by up to an order of magnitude in protein turnover rates, indicating that a primary mechanism of turnover regulation originates from agents that act on the individual substrate level, as opposed to the global proteolytic machinery of the tissue in question. Proteins with close functional relationship often share in expression regulation (96). It is pertinent to ask whether a coordinated system also exists for degradation within mitochondria, especially in light of the greater stability of mitochondrial proteins over cytosolic proteins, which would suggest they are exposed to different protein degradation mechanisms. What, if any, physiological functions or adaptive advantage the slower turnover of mitochondrial proteins provides is not clear. It is generally assumed that abundant housekeeping and structural proteins have longer half-lives, whereas regulatory enzymes or signaling molecules tend to have shorter half-lives. This arrangement is thought to allow quick establishment of new steady-state concentrations for regulatory proteins in response to rapid changes in the environment. The stability of mitochondrial proteins therefore seems paradoxical given the need for constant regulation of critical metabolic enzymes that reside inside the organelle. Interestingly, large-scale studies have generally demonstrated that turnover rates in examined mitochondrial proteins are not primarily controlled by previously proposed sequence features (PEST region, etc.), whereas the bacterial homolog of LONP1 is thought to recognize degrons on the substrates (97). Whether there are underlying rules to the individual turnover rates of proteins in the mitochondrion will be an interesting subject of investigation.

3.3 Physiological consequences of temporal dynamics

As stated above, protein temporal dynamics can change drastically in disease models or other physiological perturbations, including isoproterenol-mediated cardiac remodeling (68), transverse aortic constriction (70), and caloric restriction (98). Mitochondrial proteins that have been identified with altered turnover rates in heart diseases are potential targets for therapeutics. Our group examined the kinetic signatures of murine proteins during adverse cardiac remodeling and found the diseased heart to be associated with global alterations in turnover dynamics. Mitochondrial proteins can show opposite response in the remodeling heart: while the turnover rate of many mitochondrial proteins was drastically escalated, others remained unvaried or even diminished in turnover (68). Thus whilst the degradation of a subset of mitochondrial may have an oversized effect on organelle dynamics, the data corroborate that mitochondrial proteins are regulated independently and highlight the importance of understanding individual protein dynamics. Further investigation may delineate the mechanisms by which mitochondrial protein turnover is involved in cardiac pathogenesis.

Notably, the spatial distribution of proteins likely plays a direct role in regulating its temporal stability, suggesting that measurements of protein turnover may be harnessed as a means to determine whether individual protein pools in multiple cellular compartments undergo rapid equilibration (Figure 4). This hypothetical method would take advantage of the observation that proteins that are targeted to the mitochondria are on average twice as stable as protein species in the cytosol [13]. In contrast to proteins that are conventionally assumed to be primarily localized to the mitochondrion, some proteins that are known to reside in multiple compartments do in fact show differential turnover rates from the cytosolic and mitochondrial preparations, which may reflect compartment-specific turnover. For instance, in addition to forming the gap junctions at the intercalated disks between two adjacent cardiomyocytes, connexin 43 (Cx43/GJA1) also localizes to cardiac mitochondria where the protein forms hemichannels on the mitochondrial inner membrane and potentially contributes to mitochondrial K^+ influx [50]. In previous experiments by our group, we labeled total cardiac proteins with 2H_2O labeling in mice, then isolated cardiac mitochondrial and mitochondria-depleted cytosolic proteins for independent analysis. We observed that the cytosolic population of Cx43 turns over significantly faster than the mitochondrial Cx43 population [13]. The mitochondrial and cytosolic populations also altered in distinct manners in a cardiac hypertrophy model, with the cytosolic subpopulation being up-regulated at 1.22-fold, but the mitochondrial subpopulation being up-regulated 1.60-fold [13], which indicate the two Cx43 pools were differentially regulated in hypertrophy. More importantly, from these temporal dynamics data one may infer that the cytosolic and mitochondrial populations of Cx43 are in disequilibrium in the heart, as otherwise the measured isotope incorporation rate would be identical and reflect an combination of turnover processes in the involved cellular compartments. This is in contrast to the case of the glycolytic protein isoforms HKI and HKII for which the subpopulations appear to constantly exchange. In cardiac cells, HKI is thought to be primarily stably located on the mitochondrial outer membrane surface, and has a turnover rate that is typical of a mitochondrial protein, approaching the average of all mitochondrial proteins whether when measured from mitochondrial preparations or mitochondria-depleted cytosolic fractions.

HKII, on the other hand, is known to translocate dynamically between the mitochondrial surface and cytosol according to glucose metabolic status as discussed above (51). Consistent with this, HKII in cardiac cells exhibit turnover rates that are typical of a cytosolic protein whether measured from either mitochondrial or mitochondria-depleted cytosolic preparations.

4. Is mitochondrial protein dynamics clinically relevant?

We offer here some prospective opinions on the outlook of clinical translation of mitochondrial proteomics. The potential benefits of mitochondrial protein biomarkers are easy to envision. Efforts to characterize the cardiac mitochondrial proteome have led to increasing evidence on critical mitochondrial perturbations in cardiovascular diseases. Disease-associated changes in protein dynamics will further add to the potential for clinical translation. Nevertheless, the utility of these mitochondrial proteins has yet to be translated to clinical markers. Two challenges that are peculiar to the translation of mitochondrial protein dynamics are the difficulty in sampling disease-relevant mitochondrial proteins in clinical specimens, and the time-scale required for temporal dynamics studies in human.

Where a primary mitochondrial lesion is concerned, such as in a disease due to genetic differences in mitochondrial DNA, mitochondrial proteins may be extracted and examined from direct muscle biopsy (99). These approaches however are not generally applicable for acquired cardiovascular diseases, as muscle mitochondria may not present identical alterations as those in the heart, and direct biopsies of cardiac samples are overly invasive in most situations. On the other hand, peripheral blood samples provide a non-invasive source of biomarkers for the diagnosis and prognosis of complex diseases, but mitochondria are only found in monocytes in peripheral blood, which are likewise not necessarily indicative of the status of the mitochondrial proteome in the heart. An obvious obstacle here is that mitochondrial proteins are not conventionally thought to be present in the plasma, and the plasma contains an admixture of proteomes from different tissue leakages. Nevertheless, proteomics data suggest that the human plasma contains effluent from most human organs and mitochondrial proteins can be identified from plasma samples. The Plasma Proteome Database lists more than half of human proteins coded by the genome (10,546 entries as of the 2014 update (100), with multiple mitochondrial proteins including most TCA cycle enzymes, as well as ATP synthase subunits. The difficulty then is whether one can differentiate the tissue origin of any circulating mitochondrial proteins, or whether the discovered proteins are simply contaminants from blood cells, or simply “moonlighting” in other cellular compartments.

Future solutions to this quandary may lie in the discovery of exosomal proteins from cardiac myocytes. Exosomes are phospholipid vesicles that are 20 to 100 nanometers in diameters that are shredded from different cell types and that may be isolated from bodily fluids including plasma, urine, and saliva using ultracentrifugation or immunoaffinity (101–103). Exosomes are thought to be a cell-to-cell communication mechanism, and contain miRNA, mRNA, and a subset protein as cargoes, which can influence physiological functions upon fusion with the target cells (104). Human mesenchymal stem cells secrete exosomes that when transported to mouse models of ischemia/reperfusion injury are able to reduce infarct

size (105), suggesting cardiac physiology is receptive to, and participates, in exosome biology.

Exosomes are generated by a double invagination process that preserves membrane topology. Hence, the surface of exosomes may contain cell surface protein markers that may allow for the isolation of tissue-specific exosomes for targeted investigations (i.e., of only cardiac cargoes in circulation). Proteomics experiments on exosome composition have revealed both a subset of universal exosome markers, as well as tissue-of-origin fingerprints. Cardiac exosomes, shredded from cultured cardiomyocytes, include released 60 kDa heat shock protein (HSP60), HKP70, HSP90 as well as a number of mitochondrial proteins found such as HSP60, VCP, malate dehydrogenase, isocitrate dehydrogenase, and ATP synthase (106, 107). It is likely additional exosome proteins remain to be discovered, as exosomes from non-cardiac cell types have been found to contain no less than 300 distinct protein species (106). If suitable markers can be found to avail the isolation of cardiac exosomes from circulating biofluids, the isotope signatures of these proteins may prove useful in the quest to identify markers for cardiovascular diseases based on their temporal dynamics.

Having thus stated, a current challenge is that the detection of minute amounts of proteins remains problematic, especially in tissues with high dynamic range of protein concentration such as the plasma. This difficulty is compounded in protein dynamics studies, which generally present a higher barrier of sensitivity to deducing useful information than protein identification experiments. Existing studies of protein turnover dynamics in human plasma including those from our group have generally only quantified with confidence the top 100–500 most abundant proteins (108, 109). Any exosomal or cell-free mitochondrial proteins in the plasma are likely to be low in relative abundance in comparison to the abundant plasma proteins, which would limit the ability to effectively analyze the proteomic properties of cardiac mitochondria from circulating samples.

Lastly, the presumably long half-life of human cardiac proteins will also limit experimental designs, such as the minimal duration for which a human subject must be labeled in order for sufficient stable isotopes to be incorporated in cardiac proteins for their turnover rates to be determined. An average mouse plasma protein has a half-life of roughly two days, whereas an average cardiac mitochondrial protein has a seven-fold longer half-life, at approximately two weeks. Given that an average human plasma protein has half-life that is again twice as long as that of a mouse's, the half-life of an average mitochondrial protein in the human heart may be as long as four weeks. Hence some logistic challenges will need to be addressed in order for one to label human subjects for prolonged periods, or alternatively, initial experiments will need to be restricted to the measurement of fast-turnover proteins.

Expert commentary

A distinction between the proteome and the genome is the large number of properties that appear to be simultaneously required to adequately describe the anatomy and function of a protein pool. The proteome has been compared to the genome as a house to a floor plan (110), but initial proteomics studies scarcely ventured beyond identifying and quantifying the catalog of proteins in the cell, and only recently have the minutiae of the house begun to be elucidated. From whole-organelle fusion-fission cycles to protein complex formation,

spatiotemporal dynamics can be seen as a manifestation of a fundamental property of the proteome, namely that in order for the cellular proteome to function correctly, its component must appear in the right place at the right time. In other words, the function and functionality of a protein are dictated by its spatial and temporal distributions in the cell, properties which cannot be deduced from static protein profiles, let alone from transcript data. It appears all but certain that the underlying rules of cellular regulations has evolved to subserve such an architecture, as proteins that share in assumed biological functions would be assumed to share also in their intracellular localization. Less obviously, they also tend to share in their physiological stability, even in the absence of obvious sequence homology.

Five-year view

Increasing sophistication in instrumentation and study designs are driving the coverage and diversity of proteomics experiments. Discovery experiments will soon reach sufficient sensitivity to identify proteins that are present in as few as one copy per cardiac mitochondrion (~1 fmol, assuming 50 µg loading and 50 fg mitochondrial protein mass (111)). Benefitting from increased proteomics performance, protein dynamics studies will further shed light on protein crosstalk, equilibrium, and their pathological implications that are beyond the reach of static expression profiling studies. In the near future diseased states may be more directly described as spatial and temporal distributions having gone awry, in addition to the genetic response of the cell to alter the expression level of proteins. With the accumulation of ever-growing data volume, size, and complexity, however, one can foresee data science challenges to be a new area of opportunities in the analysis of proteomics data. High-throughput computational and tools platforms over the next five years will be crucial to the discovery and utility of large, high-dimensionality, and heterogeneous datasets. Already a number of bioinformatics platforms are being launched to aggregate omics data with information from various organelles and disease models that currently reside in fragmented databases (112–114). At the same time, translational applications of mitochondrial dynamics will remain a topic of heavy exploration. As our knowledge on mitochondrial proteins continues to grow in animal models, the knowledge gap in interpreting and translating mitochondrial proteomic data to the clinic will become more apparent. The need for effective translational strategies will compound and call for initiatives that bring together clinical researchers and proteomics method developers in sustained and mutually beneficial collaborative endeavors.

References

Papers of special note have been highlighted as:

* of interest

** of considerable interest

1. Ventura-Clapier R, Garnier A, Veksler V. Energy metabolism in heart failure. *J Physiol.* 2004; 555:1–13. [PubMed: 14660709]
2. Rosca MG, Hoppel CL. Mitochondria in heart failure. *Cardiovasc Res.* 2010; 88:40–50. [PubMed: 20668004]
3. Abel ED, Doenst T. Mitochondrial adaptations to physiological vs. pathological cardiac hypertrophy. *Cardiovasc Res.* 2011; 90:234–242. [PubMed: 21257612]

4. Rosca MG, Hoppel CL. Mitochondrial dysfunction in heart failure. *Hear Fail Rev.* 2012
5. Balaban RS. The mitochondrial proteome: A dynamic functional program in tissues and disease states. *Environ Mol Mutagen.* 2010; 51:352–359. [PubMed: 20544878]
6. Lotz C, Lin AJ, Black CM, Zhang J, Lau E, Deng N, Wang Y, Zong N, Choi J, Tao X, Liem D, Korge P, Weiss JN, Hermjakob H, Yates JR 3rd, Apweiler R, Ping P. The Characterization, Design, and Function of the Mitochondrial Proteome: From Organs to Organisms. *J Proteome Res.* 2013 Epub DOI.
7. Hammerling BC, Gustafsson ÅB. Mitochondrial Quality Control in the Myocardium: Cooperation between Protein Degradation and Mitophagy. *J Mol Cell Cardiol.* 2014; 75:122–130. [PubMed: 25086292]
8. Gottlieb RA, Gustafsson AB. Mitochondrial turnover in the heart. *Biochim Biophys Acta.* 2011; 1813:1295–1301. [PubMed: 21147177]
9. Dorn GW 2nd. Mitochondrial dynamics in heart disease. *Biochim Biophys Acta.* 2013; 1833:233–241. [PubMed: 22450031]
10. Liu X, Weaver D, Shirihai O, Hajnóczky G. Mitochondrial “kiss-and-run”: interplay between mitochondrial motility and fusion-fission dynamics. *EMBO J.* 2009; 28:3074–3089. [PubMed: 19745815]
11. Muster B, Kohl W, Wittig I, Strecker V, Joos F, Haase W, Bereiter-Hahn J, Busch K. Respiratory chain complexes in dynamic mitochondria display a patchy distribution in life cells. *PLoS One.* 2010; 5
12. Wikstrom JD, Twig G, Shirihai OS. What can mitochondrial heterogeneity tell us about mitochondrial dynamics and autophagy? *Int J Biochem Cell Biol.* 2009; 41:1914–1927. [PubMed: 19549572]
13. Zhang J, Lin A, Powers J, Lam MP, Lotz C, Liem D, Lau E, Wang D, Deng N, Korge P, Zong NC, Cai H, Weiss J, Ping P. Perspectives on: SGP symposium on mitochondrial physiology and medicine: mitochondrial proteome design: from molecular identity to pathophysiological regulation. *J Gen Physiol.* 2012; 139:395–406. [PubMed: 22641634]
14. Gucsek M, Murphy E. What can we learn about cardioprotection from the cardiac mitochondrial proteome? *Cardiovasc Res.* 2010; 88:211–218. [PubMed: 20805096]
15. Foster DB, O'Rourke B, Van Eyk JE. What can mitochondrial proteomics tell us about cardioprotection afforded by preconditioning? *Expert Rev Proteomics.* 2008; 5:633–636. [PubMed: 18937553]
16. Dai DF, Hsieh EJ, Liu Y, Chen T, Beyer RP, Chin MT, MacCoss MJ, Rabinovitch PS. Mitochondrial proteome remodelling in pressure overload-induced heart failure: the role of mitochondrial oxidative stress. *Cardiovasc Res.* 2012; 93:79–88. [PubMed: 22012956]
17. Foster DB, Ho AS, Rucker J, Garlid AO, Chen L, Sidor A, Garlid KD, O'Rourke B. Mitochondrial ROMK channel is a molecular component of mitoKATP. *Circ Res.* 2012; 111:446–454. [PubMed: 22811560]
18. Forner F, Foster LJ, Campanaro S, Valle G, Mann M. Quantitative proteomic comparison of rat mitochondria from muscle, heart, and liver. *Mol Cell Proteomics.* 2006; 5:608–619. [PubMed: 16415296]
19. Breckels LM, Gatto L, Christoforou a, Groen aJ, Lilley KS, Trotter MWB. The effect of organelle discovery upon sub-cellular protein localisation. *J Proteomics.* 2013; 88:129–40. [PubMed: 23523639]
- 20*. Gatto L, Breckels LM, Burger T, Nightingale DJH, Groen AJ, Campbell C, Mulvey CM, Christoforou A, Ferro M, Lilley KS. A foundation for reliable spatial proteomics data analysis. *Mol Cell Proteomics.* 2014; 13:1937–1952. Describes a general analytical model to discern spatial distribution and dynamics in continuous density gradient experiments. [PubMed: 24846987]
21. Yan W, Hwang D, Aebersold R. Quantitative proteomic analysis to profile dynamic changes in the spatial distribution of cellular proteins. *Methods Mol Biol.* 2008; 432:389–401. [PubMed: 18370032]
22. Foster LJ, de Hoog CL, Zhang Y, Zhang Y, Xie X, Mootha VK, Mann M. A Mammalian Organelle Map by Protein Correlation Profiling. *Cell.* 2006; 125:187–199. [PubMed: 16615899]

- 23**. Baqader NO, Radulovic M, Crawford M, Stoeber K, Godovac-Zimmermann J. Nuclear Cytoplasmic Trafficking of Proteins is a Major Response of Human Fibroblasts to Oxidative Stress. *J Proteome Res.* 2014; 13:4398–4423. [PubMed: 25133973]
24. Ben-Menachem R, Tal M, Shadur T, Pines O. A third of the yeast mitochondrial proteome is dual localized: A question of evolution. *Proteomics.* 2011; 11:4468–4476. [PubMed: 21910249]
25. Qattan AT, Radulovic M, Crawford M, Godovac-Zimmermann J. Spatial distribution of cellular function: The partitioning of proteins between mitochondria and the nucleus in MCF7 breast cancer cells. *J Proteome Res.* 2012; 11:6080–6101. [PubMed: 23051583]
26. Franklin S, Zhang MJ, Chen H, Paulsson AK, Mitchell-Jordan SA, Li Y, Ping P, Vondriska TM. Specialized compartments of cardiac nuclei exhibit distinct proteomic anatomy. *Mol Cell Proteomics.* 2011; 10:M110000703.
27. Choi Y-S, Hoon Jeong J, Min H-K, Jung H-J, Hwang D, Lee S-W, Kim Pak Y. Shot-gun proteomic analysis of mitochondrial D-loop DNA binding proteins: identification of mitochondrial histones. *Mol Biosyst.* 2011; 7:1523–1536. [PubMed: 21359316]
28. Walls KC, Coskun P, Gallegos-Perez JL, Zadourian N, Freude K, Rasool S, Blurton-Jones M, Green KN, LaFerla FM. Swedish Alzheimer mutation induces mitochondrial dysfunction mediated by HSP60 mislocalization of Amyloid Precursor Protein (APP) and beta-amyloid. *J Biol Chem.* 2012; 287:30317–30327. [PubMed: 22753410]
29. Iijima-Ando K, Hearn SA, Shenton C, Gatt A, Zhao L, Iijima K. Mitochondrial mislocalization underlies Abeta42-induced neuronal dysfunction in a Drosophila model of Alzheimer's disease. *PLoS One.* 2009; 4:e8310. [PubMed: 20016833]
30. Ferreira Lacerda A, Hartjes E, Brunetti CR. LITAF mutations associated with Charcot-Marie-Tooth disease 1C show mislocalization from the late endosome/lysosome to the mitochondria. *PLoS One.* 2014; 9
31. DuBoff B, Götz J, Feany MB. Tau Promotes Neurodegeneration via DRP1 Mislocalization In Vivo. *Neuron.* 2012; 75:618–632. [PubMed: 22920254]
32. Okreglak V, Walter P. The conserved AAA-ATPase Msp1 confers organelle specificity to tail-anchored proteins. *Proc Natl Acad Sci.* 2014; 111:8019–8024. [PubMed: 24821790]
33. Chen Y, Umanah GKE, Dephoure N, Andrabi SA, Gygi SP, Dawson TM, Dawson VL, Rutter J. Msp1/ATAD1 maintains mitochondrial function by facilitating the degradation of mislocalized tail_anchored proteins. *EMBO J.* 2014; 33:1548–1564. [PubMed: 24843043]
34. Xing Y, Xu Q, Lee C. Widespread production of novel soluble protein isoforms by alternative splicing removal of transmembrane anchoring domains. *FEBS Lett.* 2003; 555:572–578. [PubMed: 14675776]
35. Mancuso G, Barth E, Crivello P, Rugarli EI. Alternative splicing of Spg7, a gene involved in hereditary spastic paraplegia, encodes a variant of paraplegin targeted to the endoplasmic reticulum. *PLoS One.* 2012; 7
36. Tammineni P, Anugula C, Mohammed F, Anjaneyulu M, Lerner AC, Sepuri NBV. The import of the transcription factor STAT3 into mitochondria depends on GRIM-19, a component of the electron transport chain. *J Biol Chem.* 2013; 288:4723–4732. [PubMed: 23271731]
37. Boengler K, Hilfiker-Kleiner D, Heusch G, Schulz R. Inhibition of permeability transition pore opening by mitochondrial STAT3 and its role in myocardial ischemia/reperfusion. *Basic Res Cardiol.* 2010; 105:771–785. [PubMed: 20960209]
38. Ping P, Song C, Zhang J, Guo Y, Cao X, Li RCX, Wu W, Vondriska TM, Pass JM, Tang XL, Pierce WM, Bolli R. Formation of protein kinase C ϵ -Lck signaling modules confers cardioprotection. *J Clin Invest.* 2002; 109:499–507. [PubMed: 11854322]
39. Pagliarini DJ, Calvo SE, Chang B, Sheth SA, Vafai SB, Ong SE, Walford GA, Sugiana C, Boneh A, Chen WK, Hill DE, Vidal M, Evans JG, Thorburn DR, Carr SA, Mootha VK. A Mitochondrial Protein Compendium Elucidates Complex I Disease Biology. *Cell.* 2008; 134:112–123. [PubMed: 18614015]
40. Budas GR, Churchill EN, Disatnik MH, Sun L, Mochly-Rosen D. Mitochondrial import of PKC is mediated by HSP90: A role in cardioprotection from ischaemia and reperfusion injury. *Cardiovasc Res.* 2010; 88:83–92. [PubMed: 20558438]

41. Voos W. Chaperone-protease networks in mitochondrial protein homeostasis. *Biochim Biophys Acta - Mol Cell Res.* 2013; 1833:388–399.
42. Boengler K, Gres P, Cabestrero A, Ruiz-Meana M, Garcia-Dorado D, Heusch G, Schulz R. Prevention of the ischemia-induced decrease in mitochondrial Tom20 content by ischemic preconditioning. *J Mol Cell Cardiol.* 2006; 41:426–430. [PubMed: 16828795]
43. Baseler WA, Dabkowski ER, Jagannathan R, Thapa D, Nichols CE, Shepherd DL, Croston TL, Powell M, Razunguzwa TT, Lewis SE, Schnell DM, Hollander JM. Reversal of mitochondrial proteomic loss in Type 1 diabetic heart with overexpression of phospholipid hydroperoxide glutathione peroxidase. *Am J Physiol Regul Integr Comp Physiol.* 2013; 304:R553–65. [PubMed: 23408027]
44. Baseler WA, Dabkowski ER, Williamson CL, Croston TL, Thapa D, Powell MJ, Razunguzwa TT, Hollander JM. Proteomic alterations of distinct mitochondrial subpopulations in the type 1 diabetic heart: contribution of protein import dysfunction. *Am J Physiol Regul Integr Comp Physiol.* 2011; 300:R186–R200. [PubMed: 21048079]
45. Van Empel VPM, Bertrand ATA, Hofstra L, Crijns HJ, Doevendans PA, De Windt LJ. Myocyte apoptosis in heart failure. *Cardiovasc Res.* 2005; 67:21–29. [PubMed: 15896727]
46. Liu H-R, Gao E, Hu A, Tao L, Qu Y, Most P, Koch WJ, Christopher TA, Lopez BL, Alnemri ES, Zervos AS, Ma XL. Role of Omi/HtrA2 in Apoptotic Cell Death After Myocardial Ischemia and Reperfusion. *Circ.* 2005; 111:90–96.
47. Patterson SD, Spahr CS, Daugas E, Susin SA, Irinopoulou T, Koehler C, Kroemer G. Mass spectrometric identification of proteins released from mitochondria undergoing permeability transition. *Cell Death Differ.* 2000; 7:137–144. [PubMed: 10713728]
48. Saita S, Shirane M, Nakayama KI. Selective escape of proteins from the mitochondria during mitophagy. *Nat Commun.* 2013; 4:1410. [PubMed: 23361001]
49. Calmettes G, Ribalet B, John S, Korge P, Ping P, Weiss JN. Hexokinases and cardioprotection. *J Mol Cell Cardiol.* 2015; 78:107–115. [PubMed: 25264175]
50. Calmettes G, John Sa, Weiss JN, Ribalet B. Hexokinase-mitochondrial interactions regulate glucose metabolism differentially in adult and neonatal cardiac myocytes. *J Gen Physiol.* 2013; 142:425–36. [PubMed: 24081983]
51. John S, Weiss JN, Ribalet B. Subcellular localization of hexokinases I and II directs the metabolic fate of glucose. *PLoS One.* 2011; 6
52. Howden R. Nrf2 and cardiovascular defense. *Oxid Med Cell Longev.* 2013
53. Li J, Ichikawa T, Villacorta L, Janicki JS, Brower GL, Yamamoto M, Cui T. Nrf2 protects against maladaptive cardiac responses to hemodynamic stress. *Arterioscler Thromb Vasc Biol.* 2009; 29:1843–1850. [PubMed: 19592468]
54. Lo SC, Hannink M. PGAM5 tethers a ternary complex containing Keap1 and Nrf2 to mitochondria. *Exp Cell Res.* 2008; 314:1789–1803. [PubMed: 18387606]
55. Zhang J, Liem DA, Mueller M, Wang Y, Zong C, Deng N, Vondriska TM, Korge P, Drews O, MacLellan WR, Honda H, Weiss JN, Apweiler R, Ping P. Altered Proteome Biology of Cardiac Mitochondria Under Stress Conditions. *J Proteome Res.* 2008; 7:2204–2214. [PubMed: 18484766]
56. Heo JM, Livnat-Levanon N, Taylor EB, Jones KT, Dephore N, Ring J, Xie J, Brodsky JL, Madeo F, Gygi SP, Ashrafi K, Glickman MH, Rutter J. A stress-responsive system for mitochondrial protein degradation. *Mol Cell.* 2010; 40:465–480. [PubMed: 21070972]
57. Azzu V, Brand MD. Degradation of an intramitochondrial protein by the cytosolic proteasome. *J Cell Sci.* 2010; 123:578–585. [PubMed: 20103532]
58. Azzu V, Mookerjee SA, Brand MD. Rapid turnover of mitochondrial uncoupling protein 3. *Biochem J.* 2010; 426:13–17. [PubMed: 19954423]
59. Clarke KJ, Adams AE, Manzke LH, Pearson TW, Borchers CH, Porter RK. *Biochimica et Biophysica Acta - Bioenergetics.* 2012:1759–1767.
60. Neuspiel M, Schauss AC, Braschi E, Zunino R, Rippstein P, Rachubinski RA, Andrade-Navarro MA, McBride HM. Cargo-Selected Transport from the Mitochondria to Peroxisomes Is Mediated by Vesicular Carriers. *Curr Biol.* 2008; 18:102–108. [PubMed: 18207745]
- 61*. Soubannier V, McLelland GL, Zunino R, Braschi E, Rippstein P, Fon EA, McBride HM. A vesicular transport pathway shuttles cargo from mitochondria to lysosomes. *Curr Biol.* 2012;

- 22:135–141. Describes the transport of selected cargo from mitochondria-derived vesicles to lysosomes as a mechanism of retrotranslocation and mitochondrial quality control. [PubMed: 22226745]
62. Saada A, Edvardson S, Shaag A, Chung WK, Segel R, Miller C, Jallas C, Elpeleg O. Combined OXPHOS complex I and IV defect, due to mutated complex I assembly factor C20ORF7. *J Inher Metab Dis*. 2012; 35:125–131. [PubMed: 21607760]
 63. Li Y, D'Aurelio M, Deng JH, Park JS, Manfredi G, Hu P, Lu J, Bai Y. An assembled complex IV maintains the stability and activity of complex I in mammalian mitochondria. *J Biol Chem*. 2007; 282:17557–17562. [PubMed: 17452320]
 64. Dudkina NV, Kudryashev M, Stahlberg H, Boekema EJ. Interaction of complexes I, III, and IV within the bovine respirasome by single particle cryoelectron tomography. *Proc Natl Acad Sci U S A*. 2011; 108:15196–15200. [PubMed: 21876144]
 65. Lazarou M, Thorburn DR, Ryan MT, McKenzie M. Assembly of mitochondrial complex I and defects in disease. *Biochim Biophys Acta - Mol Cell Res*. 2009; 1793:78–88.
 66. Mimaki M, Wang X, McKenzie M, Thorburn DR, Ryan MT. Understanding mitochondrial complex I assembly in health and disease. *Biochim Biophys Acta*. 2012; 1817:851–62. [PubMed: 21924235]
 67. Moreno-Lastres D, Fontanesi F, García-Consuegra I, Martín MA, Arenas J, Barrientos A, Ugalde C. Mitochondrial complex I plays an essential role in human respirasome assembly. *Cell Metab*. 2012; 15:324–335. [PubMed: 22342700]
 - 68*. Lam MP, Wang D, Lau E, Liem D, Kim AK, Ng DC, Liang X, Bleakley BJ, Liu C, Tabaraki JD, Cadeiras M, Wang Y, Deng M, Ping P. Protein kinetic signatures of the remodeling heart following isoproterenol stimulation. *J Clin Invest*. 2014; 124:1734–1744. One of the first studies of in vivo protein temporal dynamics in a heart disease model, revealing several turnover changes in cardiac mitochondrial proteins. [PubMed: 24614109]
 69. Kasumov T, Dabkowski ER, Shekar KC, Li L, Ribeiro RF Jr, Walsh K, Previs SF, Sadygov RG, Willard B, Stanley WC. Assessment of Cardiac Proteome Dynamics with Heavy Water: Slower Protein Synthesis Rates in Interfibrillar than Subsarcolemmal Mitochondria. *Am J Physiol Hear Circ Physiol*. 2013
 70. Shekar KC, Li L, Dabkowski ER, Xu W, Ribeiro RF, Hecker PA, Recchia Fa, Sadygov RG, Willard B, Kasumov T, Stanley WC. 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. [PubMed: 24995939]
 71. Ngo JK, Pomatto LCD, Davies KJA. Upregulation of the mitochondrial Lon Protease allows adaptation to acute oxidative stress but dysregulation is associated with chronic stress, disease, and aging. *Redox Biol*. 2013; 1:258–264. [PubMed: 24024159]
 72. Smith CK, Baker TA, Sauer RT. Lon and Clp family proteases and chaperones share homologous substrate-recognition domains. *Proc Natl Acad Sci U S A*. 1999; 96:6678–6682. [PubMed: 10359771]
 73. Kaser M, Langer T. Protein degradation in mitochondria. *Semin Cell Dev Biol*. 2000; 11:181–190. [PubMed: 10906275]
 74. Bota DA, Van Remmen H, Davies KJ. Modulation of Lon protease activity and aconitase turnover during aging and oxidative stress. *FEBS Lett*. 2002; 532:103–106. [PubMed: 12459471]
 75. Bota DA, Davies KJA. Lon protease preferentially degrades oxidized mitochondrial aconitase by an ATP-stimulated mechanism. *Nat Cell Biol*. 2002; 4:674–680.
 76. Ngo JK, Davies KJ. Mitochondrial Lon protease is a human stress protein. *Free Radic Biol Med*. 2009; 46:1042–1048. [PubMed: 19439239]
 77. Ngo JK, Pomatto LCD, Bota DA, Koop AL, Davies KJA. Impairment of Lon-Induced Protection Against the Accumulation of Oxidized Proteins in Senescent Wi-38 Fibroblasts. *J Gerontol A Biol Sci Med Sci*. 2011
 78. Strauss KA, Jinks RN, Puffenberger EG, Venkatesh S, Singh K, Cheng I, Mikita N, Thilagavathi J, Lee J, Sarafianos S, Benkert A, Koehler A, Zhu A, Trovillion V, McGlincy M, Morlet T, Deardorff M, Innes AM, Prasad C, Chudley AE, Lee INW, Suzuki CK. CODAS Syndrome Is

- Associated with Mutations of LONP1, Encoding Mitochondrial AAA+ Lon Protease. *Am J Hum Genet.* 2015; 96:121–135. [PubMed: 25574826]
79. Jenkinson EM, Rehman AU, Walsh T, Clayton-Smith J, Lee K, Morell RJ, Drummond MC, Khan SN, Naeem MA, Rauf B, Billington N, Schultz JM, Urquhart JE, Lee MK, Berry A, Hanley NA, Mehta S, Cilliers D, Clayton PE, Kingston H, Smith MJ, Warner TT, Black GC, Trump D, Davis JRE, Ahmad W, Leal SM, Riazuddin S, King MC, Friedman TB, Newman WG. Perrault syndrome is caused by recessive mutations in CLPP, encoding a mitochondrial ATP-dependent chambered protease. *Am J Hum Genet.* 2013; 92:605–613. [PubMed: 23541340]
 80. Casari G, De Fusco M, Ciarmatori S, Zeviani M, Mora M, Fernandez P, De Michele G, Filla A, Coccozza S, Marconi R, Dürr A, Fontaine B, Ballabio A. Spastic paraplegia and OXPHOS impairment caused by mutations in paraplegin, a nuclear-encoded mitochondrial metalloprotease. *Cell.* 1998; 93:973–983. [PubMed: 9635427]
 81. Erjavec N, Bayot A, Gareil M, Camougrand N, Nystrom T, Friguet B, Bulteau AL. Deletion of the mitochondrial Pim1/Lon protease in yeast results in accelerated aging and impairment of the proteasome. *Free Radic Biol Med.* 2013; 56:9–16. [PubMed: 23220263]
 82. 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; 11:150–161. [PubMed: 22081942]
 83. Lau E, Wang D, Zhang J, Yu H, Lam MP, Liang X, Zong N, Kim TY, Ping P. Substrate- and isoform-specific proteome stability in normal and stressed cardiac mitochondria. *Circ Res.* 2012; 110:1174–1178. [PubMed: 22456183]
 84. Major T, von Janowsky B, Ruppert T, Mogk A, Voos W. Proteomic Analysis of Mitochondrial Protein Turnover: Identification of Novel Substrate Proteins of the Matrix Protease Pim1. *Mol Cell Biol.* 2006; 26:762–776. [PubMed: 16428434]
 85. Bender T, Lewrenz I, Franken S, Baitzel C, Voos W. Mitochondrial enzymes are protected from stress-induced aggregation by mitochondrial chaperones and the Pim1/LON protease. *Mol Biol Cell.* 2011; 22:541–554. [PubMed: 21209324]
 86. Bezawork-Geleta A, Saiyed T, Dougan DA, Truscott KN. Mitochondrial matrix proteostasis is linked to hereditary paraganglioma: LON-mediated turnover of the human flavinylation factor SDH5 is regulated by its interaction with SDHA. *FASEB J.* 2014
 87. Matsushima Y, Goto Y, Kaguni LS. Mitochondrial Lon protease regulates mitochondrial DNA copy number and transcription by selective degradation of mitochondrial transcription factor A (TFAM). *Proc Natl Acad Sci U S A.* 2010; 107:18410–18415. [PubMed: 20930118]
 88. Suzuki CK, Suda K, Wang N, Schatz G. Requirement for the yeast gene LON in intramitochondrial proteolysis and maintenance of respiration. *Science.* 1994; 264:273–276. [PubMed: 8146662]
 89. Bayot A, Gareil M, Rogowska-Wrzesinska A, Roepstorff P, Friguet B, Bulteau A-L. Identification of Novel Oxidized Protein Substrates and Physiological Partners of the Mitochondrial ATP-dependent Lon-like Protease Pim1. *J Biol Chem.* 2010; 285:11445–11457. [PubMed: 20150421]
 90. 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.
 91. Rachdaoui N, Austin L, Kramer E, Previs MJ, Anderson VE, Kasumov T, Previs SF. Measuring proteome dynamics in vivo: as easy as adding water? *Mol Cell Proteomics.* 2009; 8:2653–2663. [PubMed: 19724074]
 92. Kim TY, Wang D, Kim AK, Lau E, Lin AJ, Liem DA, Zhang J, Zong NC, Lam MP, Ping P. Metabolic labeling reveals proteome dynamics of mouse mitochondria. *Mol Cell Proteomics.* 2012; 11:1586–1594. [PubMed: 22915825]
 93. 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; 107:14508–14513. [PubMed: 20699386]
 - 94*. Claydon AJ, Beynon R. Proteome dynamics: revisiting turnover with a global perspective. *Mol Cell Proteomics.* 2012; 11:1551–1565. Reviews the principles and experimental considerations in measuring protein turnover rates in animals on a large scale. [PubMed: 23125033]

95. Chan XC, Black CM, Lin AJ, Ping P, Lau E. Mitochondrial protein turnover: methods to measure turnover rates on a large scale. *J Mol Cell Cardiol.* 2014 In Print.
96. Carmi S, Levanon EY, Eisenberg E. Efficiency of complex production in changing environment. *BMC Syst Biol.* 2009; 3:3. [PubMed: 19128449]
97. Gur E, Vishkautzan M, Sauer RT. Protein unfolding and degradation by the AAA+ Lon protease. *Protein Sci.* 2012; 21:268–278. [PubMed: 22162032]
98. Price JC, Khambatta CF, Li KW, Bruss MD, Shankaran M, Dalidd M, Floreani NA, Roberts LS, Turner SM, Holmes WE, Hellerstein MK. The effect of long term calorie restriction on in vivo hepatic proteostasis: a novel combination of dynamic and quantitative proteomics. *Mol Cell Proteomics.* 2012; 11:1801–1814. [PubMed: 22984287]
99. Larsen S, Nielsen J, Hansen CN, Nielsen LB, Wibrand F, Stride N, Schroder HD, Boushel R, Helge JW, Dela F, Hey-Mogensen M. Biomarkers of mitochondrial content in skeletal muscle of healthy young human subjects. *J Physiol.* 2012; 590:3349–60. [PubMed: 22586215]
100. Nanjappa V, Thomas JK, Marimuthu A, Muthusamy B, Radhakrishnan A, Sharma R, Ahmad Khan A, Balakrishnan L, Sahasrabudhe NA, Kumar S, Jhaveri BN, Sheth KV, Kumar Khatana R, Shaw PG, Srikanth SM, Mathur PP, Shankar S, Nagaraja D, Christopher R, Mathivanan S, Raju R, Sirdeshmukh R, Chatterjee A, Simpson RJ, Harsha HC, Pandey A, Prasad TS. Plasma Proteome Database as a resource for proteomics research: 2014 update. *Nucleic Acids Res.* 2014; 42:D959–65. [PubMed: 24304897]
101. Muller L, Hong CS, Stolz DB, Watkins SC, Whiteside TL. Isolation of biologically-active exosomes from human plasma. *J Immunol Methods.* 2014
102. Wang Z, Hill S, Luther JM, Hachey DL, Schey KL. Proteomic analysis of urine exosomes by multidimensional protein identification technology (MudPIT). *Proteomics.* 2012; 12:329–338. [PubMed: 22106071]
103. Marimpietri D, Petretto A, Raffaghello L, Pezzolo A, Gagliani C, Tacchetti C, Mauri P, Melioli G, Pistoia V. Proteome Profiling of Neuroblastoma-Derived Exosomes Reveal the Expression of Proteins Potentially Involved in Tumor Progression. *PLoS One.* 2013; 8
104. Zhao W, Zheng X-L, Zhao S-P. Exosome and its roles in cardiovascular diseases. *Heart Fail Rev.* 2014;1–12. [PubMed: 23456447]
105. Lai RC, Arslan F, Lee MM, Sze NSK, Choo A, Chen TS, Salto-Tellez M, Timmers L, Lee CN, El Oakley RM, Pasterkamp G, de Kleijn DPV, Lim SK. Exosome secreted by MSC reduces myocardial ischemia/reperfusion injury. *Stem Cell Res.* 2010; 4:214–222. [PubMed: 20138817]
106. Cosme J, Liu PP, Gramolini AO. The cardiovascular exosome: Current perspectives and potential. *Proteomics.* 2013; 13:1654–1659. [PubMed: 23526783]
- 107*. Malik, Za; Kott, KS.; Poe, aJ; Kuo, T.; Chen, L.; Ferrara, KW.; Knowlton, aa. Cardiac myocyte exosomes: stability, HSP60, and proteomics. *Am J Physiol Heart Circ Physiol.* 2013; 304:H954–65. Demonstrates that exosomal protein content is influenced by tissue origin, and that different stimuli will result in different proteins, including mitochondrial proteins, being released in exosomes from the same cell type. [PubMed: 23376832]
108. Wang D, Liem DA, Lau E, Ng DC, Bleakley BJ, Cadeiras M, Deng MC, Lam MP, Ping P. Characterization of human plasma proteome dynamics using deuterium oxide. *Proteomics Clin Appl.* 2014
109. Price JC, Holmes WE, Li KW, Floreani NA, Neese RA, Turner SM, Hellerstein MK. Measurement of human plasma proteome dynamics with (2)H(2)O and liquid chromatography tandem mass spectrometry. *Anal Biochem.* 2012; 420:73–83. [PubMed: 21964502]
110. Loscalzo J. Proteomics in Cardiovascular Biology and Medicine. *Circ.* 2003; 108:380–383.
111. Milo R, Jorgensen P, Moran U, Weber G, Springer M. BioNumbers The database of key numbers in molecular and cell biology. *Nucleic Acids Res.* 2009; 38
112. Zong N, Li H, Lam MP, Jimenez RC, Kim CS, Deng N, Kim AK, Choi JH, Zelaya I, Liem DA, Meyer D, Odeberg J, Fang C, Lu HJ, Xu T, Weiss JN, Duan H, Uhlen M, Yates J 3rd, Apweiler R, Ge J, Hermjakob H, Ping P. Integration of Cardiac Proteome Biology and Medicine by a Specialized Knowledgebase. *Circ Res.* 2013; 113:1043–1053. [PubMed: 23965338]

113. del-Toro N, Dumousseau M, Orchard S, Jimenez RC, Galeota E, Launay G, Goll J, Breuer K, Ono K, Salwinski L, Hermjakob H. A new reference implementation of the PSICQUIC web service. *Nucleic Acids Res.* 2013; 41
114. Franceschini A, Szklarczyk D, Frankild S, Kuhn M, Simonovic M, Roth A, Lin J, Minguez P, Bork P, Von Mering C, Jensen LJ. STRING v9.1: Protein-protein interaction networks, with increased coverage and integration. *Nucleic Acids Res.* 2013; 41

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Key issues

- The cardiac mitochondrion is a highly dynamic organelle, a phenomenon that extends to, and is likely underpinned by, its protein constituents. Recent proteomics advances in measuring protein localization and half-life are beginning to reveal the full extent of proteome spatiotemporal dynamics.
- The localization of proteins to more than one cellular compartment is perhaps the normal rather than the exception. Translocation of proteins in and out of mitochondria under stimuli are necessary for cardiac functions.
- During disease conditions, mitochondrial proteins can alter in stability and half-life, or differentially localize to other cellular compartments, without overt changes in overall protein abundance.
- Despite their potential for use in the clinic to diagnose and classify patients with complex diseases, these mitochondrial protein dynamics studies and indeed most mitochondrial proteomics studies at large remain underexplored in translational applications.
- Current translational prospects are restricted by the limited amount and unclear tissue origin of mitochondrial proteins in the plasma, but these may be alleviated with advances in exosome biology and increased sensitivity of proteomics techniques.

Figure 1.

Simplified schematic of the cardiac mitochondrial proteome. Major components as identified from proteomics experiments are shown, including respiratory chain/electron transport chain (ETC) subunits, fatty acid oxidation (FAO) proteins, tricarboxylic acid (TCA) cycle enzymes, proteostatic machineries (protein import, synthesis, folding, and degradation), cell death proteins, and ion channels.

Figure 2.

Basic principles of continuous gradient organelle enrichment techniques. **(A)** Cellular compartments are differentially enriched along a continuous gradient according to their buoyant density. Protein abundance values in different fractions of the density gradient are compared quantitatively using isotope-labeling or label-free mass spectrometry techniques. Certain well-validated proteins are used as organelle markers. Proteins belonging to the same organelle will show comparable quantitative profiles across all fractions. **(B)** Principal component analysis allows clusters of organelle memberships to be visualized, as in the hypothetical example shown here. Global translocations of proteins to another organelle can be traced by comparing the distribution in two samples.

Figure 3.

Potential mechanisms of protein retrotranslocation from mitochondria. Mitochondrial proteins may translocate to other organellar destinations via one of several mechanisms. **I.** Proteins tethered onto the mitochondrial outer membrane surface can detach from their anchor upon signals. **II.** A mitochondrial protein degradation pathway analogous to ERAD has been proposed, where ERAD components including VCP/cdc48 may help extract proteins including mitofusin from the organelle for degradation. **III.** Mitochondria-derived vesicles (MDVs) transport cargoes from the mitochondrion to the lysosome and the peroxisome. Known cargo proteins include MAPL to the peroxisome, and VDAC and UQCRC2 to the lysosome.

Figure 4.

Hypothetical experiment to measure mitochondrial protein turnover rates and the equilibrium of dual-localized proteins. **(A)** A dual-localized protein can be conceptualized to exist in two separate protein pools (Pool 1 and Pool 2). The two differentially localized protein pools may differ in their synthesis and import rates (K_{syn}) as well as their compartment-specific degradation rates (k_{deg}), the latter enabled by exposure to different protein degradation agents. If the two protein pools are in rapid equilibration, however, their apparent turnover rates will be similar when measured from in vivo protein turnover studies. **(B)** Isotope labeling followed by organelle enrichment can be employed to separately analyze the individual protein pools of a dual-localized protein. Their turnover rates can be independently measured in individual mass spectrometry experiments. Unequal turnover rates from the two compartments ($k_1 \neq k_2$) would suggest limited exchange between the two protein pools.