Minireview

Expanding the mitochondrial interactome

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Abstract

The integration of information on different aspects of the composition and function of mitochondria is defining a more comprehensive mitochondrial interactome and elucidating its role in a multitude of cellular processes and human disease.

Mitochondria are complex, dynamic and essential organelles in eukaryotic cells. They are remarkable structures with well-known functions, such as the production of ATP via oxidative phosphorylation and a role in apoptosis. In addition, they are now being implicated in novel cellular functions (for example, oxygen sensing, signal transduction and anti-viral mechanisms). Mitochondrial dysfunction is also increasingly being shown to be relevant in disease, age-related and environmentally induced pathology and the aging process itself [1].

Mitochondria contain a DNA genome (mtDNA), clear evidence of their past as a free-living bacterium, related to the present-day α-proteobacteria, that became engulfed in an ancestral eukaryotic cell 1.5-2 billion years ago [2]. In most eukaryotes, mtDNA now primarily encodes a small, but essential, subset of genes required for oxidative phosphorylation; for example, the human mtDNA molecule harbors 37 genes (13 mRNAs specifying oxidative phosphorlaytion subunits, 22 tRNAs and 2 rRNAs) [3]. The proteins encoded in mtDNA are expressed in the mitochondrion, but the complete mitochondrial proteome is the product of two genomes, as most mitochondrial proteins are transcribed from genes in the nucleus, translated by cytoplasmic ribosomes, and imported into the organelle to their sites of action. Interestingly, this nucleus-encoded majority includes all the proteins needed to replicate mtDNA and orchestrate its expression [3]; several of these proteins have been implicated recently in human disease. Hundreds of mutations in the mtDNA itself have also been identified as the cause of a variety of maternally inherited diseases. Furthermore, accumulation of mtDNA mutations and deletions occurs in many tissues over time and are thought to contribute to aging and age-related pathology [1].

After more than a century of intensive study, we know an enormous amount about mitochondrial structure, function and biogenesis. In the case of oxidative phosphorylation, for example, the mechanism is understood in great detail [4]. The ability of budding yeast to grow both aerobically and anaerobically (without the need for oxidative phosphorylation) was instrumental in this success [5], along with a multidisciplinary attack on the problem by a large number of investigators using the tools of genetics, biochemistry, biophysics, physiology, and cell and structural biology, as well as information from the pathology of human mitochondrial diseases.

Our understanding of mitochondrial function as a whole is still far from complete, however. Null mutations in genes required for mitochondrial protein import, for example, result in a lethal phenotype in yeast and thus cannot be studied in the same way as could the genes controlling oxidative phosphorylation. More sophisticated analyses are needed to fully define the mitochondrial proteome in yeast and other organisms, and to define those factors that do not reside in mitochondria but nonetheless affect their function. Outstanding questions include how the structural dynamics of mitochondria impact on their function, what signaling pathways regulate mitochondrial function and coordinate nuclear and mitochondrial gene expression, how mitochondrial biogenesis and activity are regulated in a tissue-specific

fashion and, last but not least, what the full impact is of mitochondrial dysfunction on human health. It is in these contexts that more recent systematic approaches are having a huge impact.

The integrative analysis of multiple datasets dealing with different aspects of mitochondria is defining novel functional relationships between genes and proteins in all aspects of mitochondrial physiology, and has also identified new mitochondrial disease loci. In a recent exemplary example of such an analysis, Lars Steinmetz and colleagues [6] have taken a machine-learning approach to construct the most comprehensive version of the mitochondrial interactome yet, using 24 complementary datasets covering various aspects of mitochondrial proteomics and genomics in yeast and other organisms. As we discuss here, their analysis will help to advance the understanding of the mitochondrial interactome on several fronts.

The integrative approach does, of course, rely heavily on high-quality individual datasets, and for mitochondria there is already a good foundation of systematic studies. Notable among these are the global analysis of protein localization in yeast using tagged open reading frames [7,8], the proteomic analysis of purified mitochondria and mitochondrial substructures using mass spectroscopy-based methods [9-13], systematic analysis of the collections of yeast gene knock-outs for mitochondrial related phenotypes [14,15], and gene-expression profiling in conditions that require mitochondrial function or when mitochondrial oxidative phosphorylation is disrupted [16-23]. Several of these studies provided critical datasets used by Perocchi *et al.* [6] in their analysis. While each of these approaches provides new and useful data, individually they can illuminate only a limited part of the whole mitochondrial system - hence the need for integrated analysis to achieve complete resolution of the mitochondrial network.

Integrative analysis has already accelerated the cataloging of mitochondria-related components and yielded new insights into the mitochondrial system and its ties to human disease, as the following few examples illustrate. Proteomic analysis of mitochondria from different mouse tissues combined with gene-expression profiling has shed light on the tissue specificity of the mitochondrial proteome in mammals and its regulation [10]. Combining gene-expression profiling and proteomic data with genetic mapping facilitated the identification of the gene *LRPPRC* as a disease locus for the mitochondrial disorder Leigh Syndrome French-Canadian type [24]; LRPPRC is thought to encode a protein involved in mitochondrial gene expression [25]. Using the known differences in the architecture of oxidative phosphorylation among model organisms, a molecular chaperone required for assembly of mitochondrial complex I has been identified as the cause of progressive encephalomyopathy in humans [26]. Through the simultaneous analysis of 8 genome-scale datasets, 1,080 genes with a high probability of being mitochondria-associated have been defined, including 368 not previously assigned as potentially relevant to mitochondrial function [27]. When combined with genetic mapping data, this information enabled the gene *MPV17* to be identified as a locus for a disease in the "mtDNA-depletion syndrome" class [28], characterized by class of human mitochondrial diseases characterized by a severe reduction in the number of mtDNA molecules in specific tissues [29].

Perocchi *et al.* [6] have now taken integrative analysis even further by combining information from 24 published datasets. They identified 895 proteins in what they call the "mitochondrial system" of budding yeast, of which 13% have a detectable α-proteobacterial ancestry and 60% have human orthologs. Of particular interest, about two-thirds of the mitochondrial proteins implicated in human disease have orthologs in this yeast mitochondrial system; many of these have a clear α-proteobacterial ancestry, a correlation that has been documented previously [30]. Perocchi *et al.* [6] point out that in many cases, deletion of the yeast ortholog of a human mitochondrial disease gene results in a relatively mild phenotypic change - rather than a lethal phenotype or the 'petite' phenotype seen when genes involved in oxidative phosphorylation are knocked out [5]. In other words, genes that are absolutely required for mitochondrial function in yeast are poorly represented among human disease loci. This is likely to be because loss-of-function mutations in the orthologous human genes are probably incompatible with development or survival in humans as well.

Using the program STRING [31], a search tool for retrieving interacting genes, Perocchi *et al.* [6] generated an extensive network of nearly 10,000 interactions. This is the most comprehensive version of the yeast mitochondrial interactome compiled so far and will advance our understanding of mitochondrial function in various ways. First, the authors were able to place groups of mitochondrial proteins into one of 164 functional modules. This not only highlighted potential novel functional interactions between known mitochondrial proteins, but will also provide a framework for testing hypotheses regarding members of the mitochondrial proteome of unknown function. Second, as well as defining interactions between mitochondrially localized proteins, the mitochondrial interactome compiled by Perrochi *et al.* [6] also implicates other cellular proteins and processes that are not confined physically to the organelle but are still critical for its function. This is not surprising, given the well-documented dependence of mitochondria on signaling pathways that connect the nucleus and mitochondria [32] and the mitochondrial requirement for building blocks such as nucleotides [33,34] and lipids that are synthesized elsewhere in the cell. The new findings should, however, provide new insights into precisely which signaling and metabolic pathways are involved and how mitochondria are regulated in concert with other cellular activities.

With the current explosion in the availability of genome-wide and systems data, the need for comprehensive integrated analysis is clear. Such combinatorial analysis will need to mine not only mitochondria-centric datasets, but also those that examine other aspects of cell physiology at a global level, as well as traditional data repositories such as disease databases, evolutionary relationships and the vast literature. The recent advances in our understanding of the mitochondrial proteome and its interactions serve as an instructive paradigm for related studies on other cellular organelles and processes. And, as we have emphasized, clues to the pathology of human disease are gained through the novel interactions and potential links to function unearthed by these methods. Perhaps most importantly, high-quality genome-scale analyses and the subsequent comprehensive mining of all available datasets help to accelerate experimental basic and biomedical research by enabling the formulation of specific hypotheses that can be tested directly using modern techniques. A successful marriage of systematic information and hands-on experimentation is the key to fully elucidating the complexities of biological systems and mechanisms of disease.

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