Pressing Mitochondrial Genetics Forward

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Mitochondria are crucial for many cellular functions. In this issue of Cell Reports, studies from Lanning et al. and Wolf and Mootha describe RNAi approaches to screening the mitochondrial proteome. Unexpectedly, they uncover key roles for two poorly characterized mitochondrial proteins: AK4 and FASTKD4. These studies provide examples of the power of forward genetic screens, even when screening a subset of genes, in deciphering functions of previously mysterious mitochondrial proteins.

The mitochondrion is a complex organelle that performs fundamental functions necessary for many aspects of cell biology, including ATP production, lipid and amino acid metabolism, biogenesis of iron-sulfur clusters, apoptosis, and a myriad of others. Indeed, failure or dysregulation of this organelle is associated with common diseases such as cancer, diabetes, and neurodegenerative disease as well as many rare diseases. As such, mitochondrial proteins are disproportionately represented among human disease genes. In spite of this, important aspects of mitochondrial function remain poorly understood. In an effort to better understand this organelle, detailed analysis of the mitochondrial proteome has been conducted in a number of species. The most complete inventory of the mammalian mitochondrial proteome, MitoCarta, includes about 1,100 nuclear-encoded mitochondrial proteins (Pagliarini et al., 2010). However, in spite of significant ongoing effort, many mitochondrial proteins remain uncharacterized and should be further explored (Schmidt et al., 2014). A number of laboratories are employing a variety of approaches to bridge this gap. In this issue of Cell Reports, two studies describe the utilization of the MitoCarta resource to identify mitochondrial proteins that are critical in maintaining cellular bioenergetics and mitochondrial RNA processing and may directly impact human health (Lanning et al., 2014; Wolf and Mootha, 2014). They also both provide resources to the community that will help others in the ongoing quest to “functionalize” the mitochondrial proteome.

Why is it important to catalog and study mitochondrial proteins that modulate cellular bioenergetics? Presumably, the major direct effectors of ATP production by oxidative phosphorylation (OXPHOS) in mitochondria are well known. However, recent advances in metabolomics and metabolic flux analyses reveal that the efficiency and flux of OXPHOS-driven ATP production responds profoundly to developmental cues and different physiological or pathological states. The Warburg effect refers to the metabolic profile adopted by many cancer cells, wherein they increase glycolysis to produce ATP despite adequate oxygen levels. Robust glucose metabolism enables some of the glycolytic intermediates to be shunted toward biomass production for cell growth and proliferation (Kroemer and Pouyssegur, 2008). It appears that a similar metabolic program is employed by most highly proliferative cells, including stem cells and other “activated” cell types (Stanley et al., 2014). Still, how this metabolic reprogramming is signaled and enacted is not fully understood. Therefore, it is of great value to determine the impact of individual mitochondrial proteins on metabolic function and plasticity. Lanning et al. (2014) developed a high-throughput assay that systematically and comprehensively measures ATP levels in response to silencing the expression of each mitochondrial protein under different growth conditions. They forced cells to use specific fuel sources to modulate their reliance on glycolysis, OXPHOS, or a combination of the two in order to maintain their bioenergetics. The authors highlight the possibility that perturbation of the electron transport chain complexes increases glycolysis-driven ATP production as a component of metabolic reprogramming (Lapuente-Brun et al., 2013). To this end, they characterized AK4, a poorly studied mitochondrial adenylate kinase that is catalytically inactive. Previous studies revealed that AK4 interacts with the mitochondrial ADP and ATP translocase under oxidative stress conditions (Liu et al., 2009). Silencing of AK4 reduces cellular ATP level by 25% and concomitantly decreases the ATP/ADP ratio, which activates the AMPK energy-sensing protein kinase. Surprisingly, Lanning et al. (2014) also observed that knockdown of AK4 promotes cell proliferation, but this is in apparent contradiction, found that the expression level of AK4 negatively correlates with glioma patient survival. Rather than catalyzing phosphate transfer like AK2 and AK3, AK4 may maintain the mitochondrial adenine nucleotide pool by sensing the mitochondrial ATP/ADP ratio and regulating the transport activity of ANTs. Future investigation is necessary to understand the underlying mechanism, but AK4 appears to be an important mitochondrial bioenergetic regulator with potential impact on human disease, having already been associated with lung cancer (Jan et al., 2012).

Wolf and Mootha (2014) also use the MitoCarta compendium to ask an important question regarding how cells coordinate expression from the mitochondrial genome with nuclear gene expression. Mitochondrial DNA (mtDNA) is transcribed in two continuous polycistrionic: the heavy and light precursor strands. Then, these are each processed in order...
to liberate individual mRNAs, tRNAs, and rRNAs (Anderson et al., 1981). Interestingly, it appears that OXPHOS complex concentration correlates with the distinct stability of mitochondrial mRNAs that encode proteins resident in that complex. However, the mechanistic underpinning of this observation is unclear. In this work, Wolf and Mootha (2014) develop a multiplexed MitoString assay to interrogate both precursor and mature mitochondrial RNA species with the nCounter Analysis System (Geiss et al., 2008). In the current study, they aimed to identify proteins that have direct effects on these RNA species. They silenced 107 genes encoding proteins with a known or predicted RNA binding domain from MitoCarta. They rediscovered a number of proteins with known or hypothesized functions in mtRNA synthesis, processing or stabilization. More interestingly, they uncovered an uncharacterized protein, FASTKD4, which plays an important role in the stabilization of a subset of mRNAs. The stability of these mRNA species may directly influence the abundances of the OXPHOS complexes with a potentially large role in determining the metabolic phenotype of the cell.

In addition to providing useful resources, both studies provide examples demonstrating the utility of phenotypic genetic screens akin to classical forward genetics for identifying new participants in mitochondrial function. Utilizing powerful RNAi or genome-editing technologies combined with a high-throughput readout of the function of interest will open new avenues in mitochondrial research. These new avenues will bridge the current gap between mitochondrial physiology and human health.

REFERENCES


