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Mitochondrial Chemical Biology: New Probes Elucidate the Secrets of the Powerhouse of the Cell

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Mitochondria are energy-producing organelles with essential functions in cell biology, and mitochondrial dysfunction is linked to a wide range of human diseases. Efforts to better understand mitochondrial biology have been limited by the lack of tools for manipulating and detecting processes occurring within the organelle. Here, we highlight recent significant advances in mitochondrial chemical biology that have produced new tools and techniques for studying mitochondria. Specifically, we focus on the development of chemical tools to perturb mitochondrial biochemistry, probes allowing precise measurement of mitochondrial function, and new techniques for high-throughput characterization of the mitochondrial proteome. Taken together, these advances in chemical biology will enable exciting new directions in mitochondrial research.

Introduction

Mitochondria are critical organelles within eukaryotic cells that perform a remarkably diverse set of cellular functions. Most prominent of these is the generation of ATP via oxidative phosphorylation, but mitochondria also play critical roles in the regulation of apoptosis, maintenance of cellular redox homeostasis, and intracellular calcium signaling (Duchen, 2004; Rizzuto et al., 2012; Sena and Chandel, 2012; Tait and Green, 2010). The structural features of this organelle reflect the importance of maintaining a tightly regulated interface with other subcellular compartments. They are enclosed by a double membrane structure comprising an outer and inner membrane. The inner membrane is highly impermeable, being composed of a densely packed and particularly hydrophobic combination of phospholipid molecules (Smith et al., 2012). Mitochondria actively maintain a highly electronegative (−140 to −180 mV) potential across this inner membrane, which is generated by protein complexes of the electron transport chain (ETC). Maintenance of this gradient requires active transport of H^+ ions from the mitochondrial matrix to the intermembrane space, which also creates a pH differential across the inner membrane. These potential and concentration gradients provide an electromotive force that powers ATP synthesis and are essential to maintaining mitochondrial function (Smith et al., 2012).

Mitochondria contain hundreds of different proteins, the vast majority of which are encoded on the nuclear genome and imported into mitochondria post-translationally (Schmidt et al., 2010). However, mitochondria also contain a small amount of endogenous DNA (mtDNA), a circular, multi-copy genome residing in the mitochondrial matrix (Kazak et al., 2012). Mammalian mtDNA encodes 13 essential subunits of the oxidative phosphorylation machinery, which are transcribed and translated within the organelle itself. Crucial components of this translational machinery, such as rRNA and tRNA molecules, are also encoded on the mitochondrial genome.

Mitochondrial dysfunction has been linked to a wide array of different diseases (Calvo and Mootha, 2010). The reactive intermediates produced by the ETC make mitochondria a potent endogenous source of reactive oxygen species (ROS), which can cause a diverse range of macromolecular damage. Excess mitochondrial ROS production has been linked to nuclear DNA damage that promotes Huntington's disease, as well as other neurological disorders (Kovtun et al., 2007; Lin and Beal, 2006). A number of inherited genetic mutations in nuclear-encoded mitochondrial genes produce severe respiratory defects and result in mitochondrial disease (Calvo and Mootha, 2010). Many such genes encode proteins of unclear function, underscoring the need for more comprehensive functional annotation of the mitochondrial proteome (Calvo and Mootha, 2010). mtDNA damage is also highly deleterious, typically leading to reduced electron transport efficiency and other metabolic defects. Mutations in mtDNA have been linked to increased rates of cancer metastasis, neurodegenerative disease, and muscular myopathies (Holt et al., 1988; Ishikawa et al., 2008; Lin and Beal, 2006; Schon et al., 2012). mtDNA mutations may even be critical in fundamental biological processes such as aging. One study, for example, showed that mice accumulating mtDNA mutations at high rates exhibit a rapid aging phenotype, although the impact of the typical (much lower) endogenous levels of mtDNA mutation observed in humans is less clear (Kujoth et al., 2005; Pinto and Moraes, 2015).

Given the importance of mitochondria in these diverse areas of human health, it is essential to better understand the biochemical processes that support mitochondrial function (Figure 1). However, the study of mitochondrial biology faces unique limitations that have held back progress in this area. The impermeability of the inner mitochondrial membrane makes processes within mitochondria uniquely refractory to manipulation with small molecules. Important parameters of mitochondrial health,

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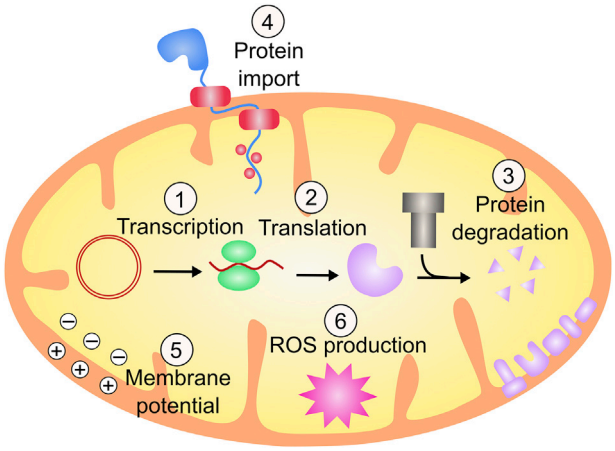

Figure 1. Potential Target Pathways for Mitochondrial Chemical Probes

(1) Mitochondria encode for specific mitochondrial genes on a multi-copy circular genome, which is replicated and transcribed by mitochondrial specific machinery. Currently, our understanding of the factors involved in mtDNA replication and repair is incomplete. (2) Mitochondrial mRNAs are translated by the mitochondrial-specific translation machinery. A better understanding of mitochondrial translation could have an impact on the development of new therapeutics. (3) Proteins marked for degradation are digested by the mitochondrial protease ClpXP; little is known about the regulation of this pathway. (4) Proteins required for mitochondrial function not encoded by mtDNA are imported by the TIM/TOM import complex. By making other systems for import available, a more versatile arsenal of probes can be developed. (5) Mitochondrial energy production and membrane polarization are mediated by the electron transport chain. While this set of pathways has been characterized extensively, new probes that can interact with the ETC could be of use therapeutically. (6) Reactive oxygen species are produced by electron leaking from the ETC; characterizing the cellular reaction to mitochondrial ROS will enable us to better characterize the mechanisms of disease that are linked with ROS production.

such as membrane potential, ROS production, and metal ion concentration, are often difficult to study in living systems. Finally, annotation of the mitochondrial proteome has historically been difficult due to the challenge of isolating highly purified mitochondria from living cells. Research characterizing new mitochondrial biology has thus been limited by a lack of robust tools and technologies for probing mitochondrial function.

In recent years, researchers from many disciplines have made significant advances toward solving these crucial problems. New chemistries have allowed the targeting of small-molecule agents specifically to mitochondria, facilitating novel ways of perturbing and assessing mitochondrial function. Novel technologies, meanwhile, are beginning to provide new avenues for the rapid, facile identification of mitochondrial proteins, illuminating a much more comprehensive picture of the mitochondrial proteome. Here, we review recent advances in chemical biology that will, in the coming years, greatly facilitate the study of mitochondria.

New Tools for Functional Manipulation of Mitochondrial Biology

Cell biology research is greatly facilitated by tools that allow for simple, specific manipulation of biochemical processes in living cell models. Mitochondria are enclosed by an outer and inner membrane, with the latter being impermeable to most molecular species. Processes occurring within mitochondria are thus particularly resistant to perturbation with small-molecule probes. Tools for modulating gene expression, such as small interfering RNA (siRNA) and clustered regularly interspaced short palindromic repeats (CRISPR)-based techniques, are similarly inapplicable to genes encoded within the organelle. In recent years, however, chemical biologists have developed new strategies for targeting biochemical pathways in mitochondria, an effort that has provided a growing toolkit of probes for investigating mitochondrial biology.

Tools for Altering the Mitochondrial Genome

The development of nuclear DNA-damaging agents has long been a focus of medicinal chemistry owing to the utility of such compounds as anticancer therapeutics (Cheung-Ong et al., 2013; Helleday et al., 2008). These compounds also provide cell biologists with useful tools for inducing well-characterized forms of DNA damage in living cells, facilitating the investigation of nuclear DNA damage and repair pathways (Chang et al., 2002; Cheung-Ong et al., 2013; Zhou et al., 2002). Most commonly used DNA-damaging agents, however, cannot penetrate the inner mitochondrial membrane or cause damage to both mtDNA and nuclear DNA, making them unsuitable for deconvoluting the mitochondrial role of proteins with functions in both compartments (Cullen et al., 2007; Fonseca et al., 2011; Yakes and Van Houten, 1997).

In recent years, a variety of successful techniques have emerged to overcome this problem by delivering small-molecule DNA-damaging agents selectively to mitochondria using molecular delivery vectors. One approach, advanced within our research group, makes use of small peptides that accumulate preferentially in mitochondria of living cells (Figure 2A). These mitochondria-penetrating peptides (MPPs) are composed of alternating cationic and hydrophobic amino acid residues (Horton et al., 2008). The positive charge of these peptides drives potential-dependent localization to mitochondria, while their hydrophobicity permits transit across the densely packed, hydrophobic inner mitochondrial membrane (Horton et al., 2008). MPPs can be easily conjugated to various types of small-molecule agents and have been used to generate mitochondria-targeted DNA-damaging agents that include mitochondria-targeted thiazole orange (mtOx), a singlet oxygen-producing molecule that oxidizes DNA; chlorambucil (mtCbl), a nitrogen mustard alkylating agent that targets both DNA and proteins; doxorubicin (mtDox), a topoisomerase II inhibitor that produces double-stranded DNA breaks; and mitochondria-targeted analog of cisplatin (mtPt), an agent that creates specific intrastrand DNA crosslinks (Chamberlain et al., 2013; Fonseca et al., 2011; Mahon et al., 2007; Wisnovsky et al., 2013). Each of these molecules has been shown in separate studies to cause damage exclusively to mtDNA, with no nuclear DNA-damaging effects. These compounds have since been used as tools to study specific deleterious effects of mtDNA damage in isolation from other cellular processes.

Triphenylphosphonium (TPP) cations have also been used extensively as a platform for mitochondria-specific delivery of small molecules (Figure 2B). TPPs effectively target mitochondria due to their delocalized positive charge and relatively high hydrophobic character, both common features of molecules with mitochondria-penetrating properties (Murphy, 2008). Being both chemically tractable and highly stable in biological systems,