Perspectives on: SGP Symposium on Mitochondrial Physiology and Medicine

Mitochondria take center stage

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Mitochondria are the ubiquitous power units of animal cells, responsible for "oxphos," the aerobic synthesis of most of the ATP that serves as the common coin of cellular metabolism. To perform their primary task of oxidative phosphorylation and to manage many secondary tasks, mitochondria use more than 1,000 proteins, of which fewer than 100 are involved directly in oxidative phosphorylation. The "secondary" tasks mitochondria perform include their own maintenance, calcium regulation and signaling, lipid synthesis, and acting as gatekeepers for programmed cell death. Catalyzed by the advances in knowledge and technology that underlie great progress in all areas of cell biology, mitochondrial physiology has been undergoing a renaissance. In this special issue of the Journal we highlight some examples of this renaissance, offering a series of Perspectives on Mitochondria. The purpose of the Perspectives in General Physiology is to provide a forum where scientific uncertainties or controversies are discussed in an authoritative, yet open manner. This series had its origin in the 65th Annual Symposium of the Society of General Physiologists on "Mitochondrial Physiology and Medicine," which took place in September 2011 in Woods Hole, MA. The meeting was organized by Shey-Shing Sheu (Thomas Jefferson University) along with a committee comprising leaders in mitochondrial research, including Robert Balaban (NHLBI), Paolo Bernardi (University of Padova), Robert Dirksen (University of Rochester), Roberta Gottlieb (San Diego State University), Gyorgy Hajnóczky (Thomas Jefferson University), and Brian O'Rourke (Johns Hopkins University). A summary of the meeting was recently published (Sheu et al., 2011).

Mitochondrial proteomes

Mitochondria contain more than 1,000 proteins, of which only 13 are encoded by mitochondrial DNA. In their Perspective, Zhang et al. summarize recent advances in mitochondrial proteomics, including quantifying "subproteomes" of the mitochondria devoted to different cellular functions. The proteomes of mitochondria in various tissues (e.g., heart, muscle, brain) differ, and proteomic variation also causes or is associated with disease. Substantial differences, for example, exist between heart and liver mitochondrial proteomes that can be directly related to tissue-specific needs. Of particular medical importance are the distinct proteomic features of mitochondria from healthy and diseased cardiac myocytes. Another important and rapidly developing aspect of mitochondrial proteomics is the characterization of posttranslational modifications, some of which are dynamic and may play critical roles in modulating oxphos function on a subsecond scale (see next section). The key challenge, as articulated by Zhang et al., will be to translate the burgeoning mitochondrial proteomic databases into resources that can be readily tapped to advance our understanding of physiological function and pathophysiology.

Metabolic homeostasis of the heart

Nothing better illustrates the amazing capacity of mitochondria to meet the energy demands of tissue than their ability to power the cardiac contraction cycle throughout life, including workload increases of up to 10-fold during surges in sympathetic activity, such as those that accompany the "fight or flight response." Mitochondria perform this feat with very little change in the steady-state concentrations of key metabolites such as ATP, ADP, P_i, and NADH. A.V. Hill (1950) first noted the challenge posed by these facts, which he called "metabolic homeostasis." In his Perspective, Robert Balaban takes up Hill's challenge. Balaban marshals evidence that posttranslational modifications of the F_1F_0 ATP synthase may underlie a 10-fold modulation of the maximum rate of ATP production ($qATP_{max}$), and that calcium in the mitochondrial matrix is a major regulator of $qATP_{max}$. These ideas comprise an important (although challenging to test) hypothesis that ties

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together several key aspects of mitochondrial physiology, including the roles of variations in matrix calcium over time and of matrix proteins regulated by calcium.

New insights from mitochondrial pH measurements

As articulated in Mitchell's chemiosmotic theory, ATP synthesis in mitochondria is driven by the proton motive force, which consists of the chemical gradient for protons (ΔpH_m) and the mitochondrial membrane potential ($\Delta\Psi_m$). Measurement of ΔpH_m has been a foundation of research in mitochondrial physiology. However, classical measurements were performed in bulk suspensions, disconnecting mitochondria from the milieu of the living cell, in which many natural and signal-dependent variations in pH_i, Ca^{2+}, and metabolites to which mitochondria are sensitive occur. Genetically encoded (optogenetic) pH probes that promise to fill many gaps in the understanding of the chemiosmotic mechanism in living cells have recently been developed. In their Perspective, Santo-Domingo and Demaurex summarize recent research with mitochondrially targeted optogenetic pH probes, and, in particular, work they have done with a very promising probe, mito-SypHer. Using this probe they have observed spontaneous increases in pH_m elevations that coincided with decreases in $\Delta\Psi_m$ in single mitochondria. Santo-Domingo and Demaurex propose that these spontaneous alkalizations of the mitochondrial matrix reflect intrinsic properties of the mitochondrial proton circuit that function to stabilize the proton motive force with opposing changes in $\Delta\Psi_m$ and ΔpH_m, and summarize the evidence that this stabilization process involves several distinct exchangers and symporters.

Superoxide flashes: Quantal production of reactive oxygen species (ROS)

Studying mitochondrial production of ROS has been challenging because of the limited methods capable of real-time monitoring of ROS levels in living cells and in situ. Recently, circularly permuted yellow fluorescent protein has been shown to specifically respond to superoxide. When targeted to the mitochondrial matrix, this probe detects bursts of superoxide production, called mitochondrial superoxide flashes (mSOFs), in individual mitochondria of intact quiescent cells. The Perspective of Wei and Dirksen provides an updated assessment of the mechanisms underlying mSOF generation and raises several controversies regarding the generation of mSOFs, including the relationship between mSOFs and pH_m transients, the molecular identity of the large pore channel that opens during a flash event, and the sequence of events between pore opening and mSOF generation. Both mSOF and pH_m signals require intact electron transport chain activities, because inhibition of any one of the electron transport complexes results in the abolition of the signals. Although future experiments will be needed to resolve the relationship between ΔpH_m transients and superoxide flashes, and their role(s) in regulating mitochondrial energy and ROS generation, both signals hold much promise in revealing dynamic details of mitochondrial function in living cells.

Mitochondrial calcium transport

The pivotal role of Ca^{2+} in regulating mitochondrial function and dysfunction is well recognized. The dogma for the mechanism of mitochondrial Ca^{2+} influx has been that it is determined primarily by the mitochondrial Ca^{2+} uniporter (MCU), whose molecular identity was recently revealed (Baughman et al., 2011; De Stefani et al., 2011). Several studies have identified additional Ca^{2+} uptake pathways, including a rapid mode of uptake and the highly conserved mitochondrial protein leucine-zipper-EF-hand–containing transmembrane protein 1 (LETM1), which exhibit different Ca^{2+} affinity, kinetics, and pharmacology from the MCU. The Perspective by O-Uchi et al. examines recent studies that have attempted to uncover the molecular identities of mitochondrial Ca^{2+} influx mechanisms using genetic manipulations, including small interfering RNA. O-Uchi et al. argue that it is reasonable to expect that different tissues may regulate mitochondrial Ca^{2+} influx in different fashions, with different combinations and expression ratios of these channels and transporters, and point to studies that will clarify the diversity of Ca^{2+} influx mechanisms, explain how these Ca^{2+} transporters are regulated by signaling molecules, and identify their distinct physiological functions and pathologies.

A hotly debated issue is whether LETM1 acts as a high affinity mitochondrial Ca^{2+} influx mechanism. The Perspective by Nowikovsky et al. summarizes current understanding of the molecular nature and of the physiology and pathophysiology of LETM1. *LETM1* encodes a mitochondrial protein conserved throughout eukaryotes, both animals and plants. Its deletion is embryonic lethal, and *LETM1* mutations underlie the seizure disorder known as Wolf–Hirschhorn syndrome. The protein was identified first as a mitochondrial K^+–H^+ antiporter, but has since been argued to function as a Ca^{2+}–H^+ antiporter by Jiang et al. (2009). Nowikovsky et al. make the case that the normal electrochemical gradients for H^+ and Ca^{2+} would not allow LETM1 to act as a Ca^{2+} influx mechanism, but rather that the driving forces under physiological conditions favor LETM1 serving as a Ca^{2+} efflux rather than influx mechanism.

Shape changing: Role of morphology in mitochondrial function

The idea that mitochondrial morphology is also a key regulator of mitochondrial function has recently gained much attention. The Perspective by Galloway and Yoon summarizes the current understanding of the interplay

between mitochondrial form and function, with a specific focus on how disturbances of this interaction lead to metabolic disease. This Perspective highlights the dynamic nature of the cross talk signaling between mitochondrial shape and metabolic activity. Well-orchestrated mitochondrial fission, fusion, and molecular motor–based movement are necessary to ensure healthy bioenergetics homeostasis.

Calcium buffering and calcium in cell death

The *JGP* is also publishing several primary articles involving research on mitochondria presented at the 2011 SGP Symposium. Two of these deal with calcium influx into mitochondria and its role in triggering cell death. In work presented in this issue, Wei et al. thoroughly characterized two distinct modes of Ca^{2+} uptake in cardiac mitochondria, "MCU_{mode1}" and "MCU_{mode2}." Mode 1 has a relatively low capacity, is engaged by small (0.1–0. 2-μM) increases in Ca^{2+}, is completely inhibited by only relatively high levels (∼1 μM) of Ruthenium red (Ru360), and triggers relatively large changes in matrix free calcium. In contrast, mode 2 is responsible for the bulk of calcium transport for external calcium in the range of 2–10 μM, is inhibited by 0.1 μM Ru360, and is associated with very small changes in matrix free calcium owing to very high buffering. The data and analyses presented in this article support the conclusion that mode 1 calcium transport functions as a signal, perhaps to dynamically modulate oxidative phosphorylation (see Perspective by Balaban), whereas mode 2 is associated with a high capacity Ca^{2+}-buffering function. The investigation of Wei et al. also indicates that the trigger for activation of the mitochondrial permeability transition pore (mPTP) is unlikely to be free matrix Ca^{2+} itself, but could be a downstream byproduct of total mitochondrial Ca^{2+} loading, a conclusion that dovetails nicely with work in another paper recently published in the *JGP*, by Seidlmayer et al. (2012).

Seidlmayer et al. (2012) quantified the inorganic polyphosphate (polyP) content of mitochondria (∼300 pmol/mg of protein) in rabbit ventricular myocytes and manipulated polyP levels by means of a mitochondrially targeted polyphosphatase. Excess accumulation of Ca^{2+} in mitochondria is a key factor in triggering mPTP opening (and subsequent apoptosis) and consequent tissue damage such as occurs during cardiac ischemia reperfusion injury. Seidlmayer et al. (2012) found that depletion of polyP inhibited mPTP opening without affecting the matrix free calcium, and put forward the hypothesis that stable complexes of calcium with polyP act as triggers for mPTP opening. This is an important advance, which make a critical link between mitochondrial calcium influx, calcium buffering by polyP, and activation of the mPTP.

Mitochondrial antioxidants and scavenging of ROS

ROS are normally generated at very low levels by the mitochondrial respiratory chain but increase under conditions of stress, and can ultimately lead to tissue damage. In this issue, Aon et al. performed experiments and theoretical analyses to assess the relative contribution of glutathione (GSH) and thioredoxin (Trx), the two principal antioxidants in mitochondria, to the scavenging of H_2O_2, a major ROS. Their results show that the reactions governing the concentrations GSH and Trx are essential to maintaining minimal emission of H_2O_2 during high level respiration, and that the reactions maintaining GSH and Trx work in close concert toward achieving this end.

Letters to the editor related to these Perspectives will be published in the September 2012 issue of the Journal. Letters to the editor should be received no later than Monday, July 16, 2012. The letters may be no longer than two printed pages (approximately six double-spaced pages) and will be subject to editorial review. They may contain no more than one figure, no more than 15 references, and no significant references to unpublished work (and may not include supplemental material). Letters should be prepared according to the Journal's instructions and can be submitted electronically at http://www.jgp.org. After the letters to the editor have been published, further responses are limited to full manuscripts.

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