

layered envelope, the cristae and the crista junctions that link the cristae to the IBM. Correct architecture is prerequisite for mitochondrial function, in particular for OXPHOS and inheritance of the mitochondrial DNA. Whereas there is a plethora of information on the mitochondrial OXPHOS complexes only little is known about the molecules that determine mitochondrial architecture. We have studied several aspects of the complexity of mitochondrial architecture. One aspect relates to the structure and function of the various molecular machines that mediate the topogenesis of newly synthesized, nuclear-encoded proteins that are imported into the mitochondria. For instance, the TOM translocase in the outer membrane and the TIM23 translocase in the inner membrane work in physical conjunction to transport proteins, at the same time, across both membranes. Thus, import of these proteins is confined to the IBM. This raises the important question as to whether there is a permanent or dynamic subcompartmentation of proteins in the various parts of the inner membrane. A largely open question in this context relates to the kinds of interactions of OM and IBM in various other transport processes, one of the most important being the translocation of lipids into the mitochondria. Another aspect regards the nature, function and molecular structure of the crista junctions and crista tips and rims. In particular the proteins that are shaping these structures are largely unknown. A number of experiments and results will be presented that provide some answers to some of these questions.

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PL. 7

Proton circuits and mitochondrial dysfunction

David G. Nicholls

Buck Institute for Age Research, California, USA

E-mail: dnicholls@buckinstitute.org

The terms mitochondrial 'function and dysfunction' are used widely in the cell biology field, generally without a precise definition of their meaning. Mitchell's chemiosmotic proton circuit, first published in 1966, provides a precise quantitative framework within which to quantify these critical parameters for the life and death of the cell. The proton circuit has units of potential (the protonmotive force, Δp) and flux (the proton current, JH^+), and these additionally allow calculation of inner membrane leak conductance, $C_m H^+$ (JH^+ per unit Δp) and power ($JH^+ \times \Delta p$). The analogy with an equivalent electrical circuit has considerable utility for visualizing and manipulating the proton circuit, and is equally applicable to isolated mitochondria and intact cells. An early application of this quantitative approach was the elucidation of the regulatable proton conductance pathway in brown adipose tissue, leading to the identification of UCP1. One observation that emerged from these studies is that 'uncoupling' is not an all-or-nothing process. Thus while a large excess of a protonophore can almost totally collapse Δp , at the critical concentration at which respiratory control is just lost Δp may be only 10–20% below its maximal State 4 value, and thermodynamically competent to maintain ATP synthesis. Until this threshold is reached Δp changes modestly as $C_m H^+$ is increased. In intact cells titration to this threshold can help to define a critical parameter of mitochondrial 'function' – the spare respiratory capacity, defined as the capacity over basal of the electron transport chain in concert with the inputting metabolic pathways to support an increase in flux in response to this imposed increase in proton conductance. With the proviso that this proton current could all be utilized by the ATP synthase in the absence of protonophore, the spare respiratory capacity provides a safety margin preventing an 'ATP crisis' during periods of maximal ATP demand, for example in neurons during

potentially excitotoxic stimulation. Mitochondrial 'dysfunction' defined as a decrease in this spare respiratory capacity has been shown in various neural preparations to greatly potentiate cell death under conditions of high energy demand.

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PL. 8

Redox-optimized mitochondrial ROS balance

Brian O'Rourke, Sonia Cortassa, Miguel A. Aon

Johns Hopkins University School of Medicine, Institute of Molecular Cardiology, Baltimore, Maryland, USA

E-mail: bor@jhmi.edu

While it is generally accepted that mitochondrial reactive oxygen species (ROS) balance depends on the both rate of single electron reduction of O_2 to superoxide ($O_2^{\cdot -}$) by the electron transport chain and the rate of scavenging by intracellular antioxidant pathways, considerable controversy exists regarding the conditions leading to oxidative stress in intact cells versus isolated mitochondria. Here, we postulate that mitochondria have been evolutionarily optimized to maximize energy output while keeping ROS overflow to a minimum by operating in an intermediate redox state. We show that at the extremes of reduction or oxidation of the redox couples involved in electron transport ($NADH/NAD^+$) or ROS scavenging ($NADPH/NADP^+$, $GSH/GSSG$), respectively, ROS balance is lost. This results in a net overflow of ROS that increases as one moves farther away from the optimal redox potential. At more reduced mitochondrial redox potentials, ROS production exceeds scavenging, while under more oxidizing conditions (e.g., at higher workloads) antioxidant defense can be compromised and eventually overwhelmed. Experimental support for this hypothesis is provided in both cardiomyocytes and in isolated mitochondria from guinea pig hearts. The model reconciles, within a single framework, observations that isolated mitochondria tend to display increased oxidative stress at high reduction potentials (and high mitochondrial membrane potential), whereas intact cardiac cells can display oxidative stress either when mitochondria become more uncoupled (i.e., low mitochondrial membrane potential) or when mitochondria are maximally reduced (as in ischemia or hypoxia). The continuum described by the model has the potential to account for many disparate experimental observations and also provides a rationale for graded physiological ROS signaling at redox potentials near the minimum.

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PL. 9

The pivotal roles of mitochondria in cancer: Warburg and beyond and encouraging prospects for effective therapies

Peter L. Pedersen*

Johns Hopkins University School of Medicine, Departments of Biological Chemistry and Center for Cell Metabolism, Baltimore, Maryland, USA

E-mail: ppederse@jhmi.edu

Tumors usurp established metabolic steps used by normal tissues for glucose utilization and ATP production that rely heavily on mitochondria and employ a route that, although involving mitochondria, includes a much greater dependency on glycolysis. First described by Otto Warburg, this aberrant phenotype becomes more pronounced with increased tumor malignancy. Thus, while maintaining their capacity for respiration, tumors "turn more parasitic" by enhancing their ability to scavenge glucose. Relying significantly on