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.

doi:10.1016/j.bbabio.2010.04.026

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_mH^+ (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_mH^+ 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.

doi:10.1016/j.bbabio.2010.04.027

PL. 8

Redox-optimized mitochondrial ROS balance

Brian O'Rourke, Sonia Cortassa, Miguel A. Aon
Johns Hopkins University School of Medicine, Institute of Molecular Cardiobiology, 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 ($O_2^{\bullet -}$) 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.

doi:10.1016/j.bbabio.2010.04.028

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

this fuel, tumors shunt their metabolic flux more toward glycolysis than normal cells, a strategy that allows for tumor survival when oxygen is limiting. Also, the resultant lactic acid poisons their extracellular environment facilitating invasion and metastasis. Significantly, tumors harness a crucial enzyme to support this destructive path — to entrap and channel glucose toward glycolysis. This enzyme is HK-2 an isoform of hexokinase. Due to many-faceted molecular features including genetic, epigenetic, transcriptional, enzymatic and sub-cellular localization to mitochondria, HK-2 facilitates and promotes the high glycolytic tumor phenotype. Thus, HK-2 represents a pivotal model gene or enzyme that tumors "select for" during tumorigenesis. In this lecture, the speaker will describe both the pivotal roles played by mitochondrial bound HK-2 and show also how the small molecule 3-bromopyruvate, an inhibitor of both HK-2 and mitochondrial function selectively eradicates such tumors while sparing normal tissues.

[*]**Dr. Saroj Mathupala,** Department of Neurological Surgery and Karmos Cancer Institute, Wayne State University, Detroit, MI, USA and **Dr. Young H. Ko,** formerly of the Department of Biological Chemistry, Johns Hopkins University, School of Medicine, Baltimore, MD, USA, and now with Cancer Cure Med. L.L.C., Owing Mills, MD USA played leading roles in this work.

Recent Review:

Mathupala SP, Ko YH, Pedersen PL (2009) *Semin. Cancer Biol.* **19**: 17–24.

doi:10.1016/j.bbabio.2010.04.029

PL.10

Import and assembly of mitochondrial proteins

Nikolaus Pfanner
Institute for Biochemistry and Molecular Biology, University of Freiburg, Germany
E-mail: nikolaus.pfanner@biochemie.unireiburg.de

Most mitochondrial proteins are synthesized as precursors on cytosolic ribosomes and imported into the organelle. The translocase of the outer membrane (TOM complex) forms the main entry gate for the majority of precursor proteins. The precursors are subsequently distributed to the four mitochondrial subcompartments. Recent studies revealed a remarkable variety of different pathways and mechanisms for protein sorting in mitochondria. (i) The presequence pathway can transport preproteins into the matrix, inner membrane and intermembrane space of mitochondria. The presequence translocase of the inner membrane (TIM23 complex) cooperates with the import motor PAM. The mitochondrial processing peptidase and further enzymes cleave the preproteins to remove the presequences and generate mature proteins. (ii) The carrier pathway directs multispanning hydrophobic proteins into the inner membrane, using the Tim9–Tim10 chaperone complex of the intermembrane space and the carrier translocase of the inner membrane (TIM22 complex). (iii) Many intermembrane space proteins are imported by a redox-regulated machinery (MIA), involving disulfide-linked intermediates between the intermembrane space receptor Mia40 and precursor proteins. (iv) Protein insertion into the mitochondrial outer membrane involves different pathways for beta-barrel proteins and alpha-helical proteins. Beta-barrel proteins are inserted into the membrane by the sorting and assembly machinery (SAM complex).

References

[1] ChacinskaA. *et al.* (2009) *Cell* **138:** 628–644.
[2] Vögtle FN *et al.* (2009) *Cell* **139:** 428–439.

doi:10.1016/j.bbabio.2010.04.030

PL. 11

Mitochondria, calcium signaling and cell death by apoptosis and autophagy

Rosario Rizzuto[1], Paolo Pinton[2], Diego De Stefani[1], Carlotta Giorgi[2], Cristina Mammucari[1], Saverio Marchi[2], Alessandro Rimessi[2], Anna Romagnoli[1], Roberta Siviero[1], Erika Zecchini[1]
[1]*Department of Biomedical Sciences, University of Padua, Italy*
[2]*Department of Experimental and Diagnostic Medicine, University of Ferrara, Italy*
E-mail: rosario.rizzuto@unipd.it

Mitochondria rapidly accumulate Ca^{2+} through a low-affinity uptake system (the mitochondrial Ca^{2+} uniporter, MCU) because they are exposed to high [Ca^{2+}] microdomains generated by the opening of ER Ca^{2+} channels. These rapid [Ca^{2+}] changes stimulate Ca^{2+}-sensitive dehydrogenases of the mitochondrial matrix, and hence rapidly upregulate ATP production in stimulated cells. Ca^{2+} also sensitizes to cell death mediators, e.g. ceramide. Accordingly, we demonstrated that Bcl-2 reduces the state of filling of ER Ca^{2+} stores, and this alteration is effective in reducing the sensitivity to apoptotic challenges. I present data on: 1) The effect on mitochondrial Ca^{2+} homeostasis of other signalling pathways involved in autophagy and apoptosis (Akt, sirt3). 2) The signalling route that links oxidative stress to the activation of p66shc, an isoform of a growth factor adapter acting as apoptotic inducer. PKCβ, activated by the oxidative challenge, induces p66shc phosphorylation, with ensuing alteration of mitochondrial structure and function. We also showed that this route is involved also in adipose differentiation of muscle-derived precursors, highlighting a novel process of utmost interest in pathophysiological conditions. 3) The molecular elements of the mitochondria–ER Ca^{2+} connection. I will discuss the role of VDAC in rapidly channelling Ca^{2+} through the outer mitochondrial membrane and the specific functions of VDAC isoforms in autophagy and apoptosis.

doi:10.1016/j.bbabio.2010.04.031

PL. 12

Mitochondrial transhydrogenase — New aspects of its physiological role

RydstromJan Rydström[1], Simon P.J. Albracht[2]
[1]*University of Gothenburg, Department of Chemistry, Biochemistry and Biophysics, Sweden*
[2]*Swammerdam Institute for Life Sciences, University of Amsterdam, The Netherlands*
E-mail: jan.rydstrom@chem.gu.se

Mitochondrial nicotinamide nucleotide transhydrogenase (Nnt) catalyzes the reduction of $NADP^+$ by NADH giving NADPH and NAD^+, driven by the electrochemical proton gradient, Δp. Together with $NADP^+$-isocitrate dehydrogenase ($NADP^+$-ICH) and $NADP^+$-dependent (decarboxylating) malic enzyme (MAEB), Nnt constitutes one of the major providers of NADPH. Knockout and inhibition studies in *C. elegans* [1] and intact heart cells [2] have indicated that, as expected and previously proposed [3], the high NADPH/$NADP^+$ ratio generated by Nnt ensures a high mitochondrial GSH/GSSG ratio through the glutathione reductase (GR) reaction (for a review, see ref [4]). Recently, the commonly used C57BL/6J mouse strain was shown to harbour a mutated NNT gene, probably introduced in the 1950´s, rendering an incomplete and inactive Nnt. This C57BL/6J mouse