

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.

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Recent Review:

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

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PL.10

Import and assembly of mitochondrial proteins

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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 multispinning 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).

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PL. 11

Mitochondria, calcium signaling and cell death by apoptosis and autophagy

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Mitochondria rapidly accumulate Ca^{2+} through a low-affinity uptake system (the mitochondrial Ca^{2+} uniporter, MCU) because they are exposed to high $[\text{Ca}^{2+}]$ microdomains generated by the opening of ER Ca^{2+} channels. These rapid $[\text{Ca}^{2+}]$ 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. PKC β , 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.

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PL. 12

Mitochondrial transhydrogenase – New aspects of its physiological role

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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

strain shows a number of interesting properties which may reflect the role of Nnt in a wildtype mouse, i.e., a glucose intolerance reflecting an insufficient insulin secretion, a several-fold higher sensitivity to MPTP, an agent that introduces a Parkinson's disease-like condition, and a low frequency of spontaneous as well as chemically induced tumors. In addition, knockout of the mitochondrial superoxide dismutase (SOD2) in the C57BL/6J background produces mice with serious cardiac disease. Most of the above observations in the C57BL/6J mice can be explained by a decreased capacity for inactivating free radicals and preventing oxidative stress by GSH-linked reactions. The reason for the lowered cancer frequency is less obvious. It is proposed that Nnt, by being linked to the Δp , in addition to maintaining a high GSH, constitutes a sensing device for estimating the quality of the mitochondria and thus the host cell. A lowered Δp leads to a lower Nnt activity, a lower NADPH/NADP⁺ ratio, a higher steady-state concentration of H₂O₂, and a higher rate of apoptosis. A lack of Nnt, as in the C57BL/6J mouse, thus favours an even higher level of H₂O₂, apoptosis and counteracts tumours. The mechanisms involved will be discussed.

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PL13

The mechanism and regulation of F-ATPases

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More than 25 high-resolution structures of mitochondrial F₁-ATPase have been determined to date. Comparison of all of the structures with each other, and examination of lattice contacts in the crystals used to solve each structure show that neither the conformations adopted by the catalytic subunits nor the occupancy of those subunits by nucleotides is influenced by lattice contacts. Therefore, the structures interpreted as representing ground and transition states depict the structures of intermediates in the catalytic cycle. In the ground state two of the catalytic sites are attached by nucleotides and the third site is unoccupied, whereas in the transition state, nucleotides occupy all three catalytic sites. Two recent structures, one of yeast F₁-ATPase inhibited with yeast inhibitor protein, IF₁, the other of the enzyme crystallized in the presence of phosphonate, appear to represent post-hydrolysis pre-product release intermediates. The current structures occupy about 20° of each of the three 120° steps in a complete 360° catalytic cycle. The lecture will discuss strategies for accessing structures that represent the “missing” part of the catalytic cycle. It will also discuss the different regulatory mechanisms of F-ATPases from mitochondria, chloroplasts and bacteria, by the inhibitor protein, by a redox switch, and possibly by the binding of ATP to the ϵ -subunit, respectively.

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SEL1

The fateful encounter of mitochondria with calcium: How did it happen?

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Indirect findings in the 1950s had indicated that mitochondria could accumulate Ca²⁺. In 1961, the phenomenon was directly demonstrated using isolated mitochondria: the uptake process was found to be driven by respiratory chain activity or by the hydrolysis of added ATP. It could be accompanied by the simultaneous uptake of inorganic phosphate, in which case precipitates of hydroxyapatite were formed in the matrix, buffering its free Ca²⁺ concentration. In conditions of cytoplasmic Ca²⁺ overload mitochondria could thus store large amounts of precipitated Ca²⁺-phosphate, permitting cells to overcome situations of Ca²⁺ emergency. Work in the 1960s established that the uptake of Ca²⁺ occurred electrophoretically on an unidentified carrier, and was released via a Na⁺/Ca²⁺ antiporter (a H⁺/Ca²⁺ release exchanger was also identified, and a permeability transition pore was later also found to mediate the efflux of Ca²⁺ from mitochondria). In the mitochondrial matrix two dehydrogenases, pyruvate dehydrogenase and phosphate phosphatase, were found to be regulated by Ca²⁺. The uptake process had very low affinity for Ca²⁺: since the bulk concentration of cytosolic Ca²⁺ is in the low to mid-nM range, it was difficult to postulate a role of mitochondria in the regulation of cell Ca²⁺. Nevertheless, energy linked Ca²⁺ transport did occur efficiently in mitochondria of various tissues in situ. The paradox was only solved in the 1990s, when it was found that the concentration of Ca²⁺ in the cytoplasm is not uniform: as perimitochondrial micropools of high Ca²⁺ concentration, sufficient to activate the low affinity uniporter, are created by the agonist-promoted discharge of Ca²⁺ from vicinal stores. Mitochondria thus regained center stage as important regulators of cytoplasmic Ca²⁺ (not only of their own internal Ca²⁺). Their Ca²⁺ transport systems react very rapidly, even in the 150–200 ms time scale of processes like the contraction and relaxation of heart. An important recent development in the area of mitochondrial Ca²⁺ transport is the involvement in the disease process. Ca²⁺ signaling defects are now gaining increasing importance in the pathogenesis of diseases, particularly in neurodegenerative diseases.

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SEL2

Wanderings in bioenergetics and biomembranes

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Having worked for 55 years in the center and at the fringe of bioenergetics, the major research stations are reviewed in my wanderings from microsomes to mitochondria, from NAD to CoQ, from reversed electron transport to reversed oxidative phosphorylation, from mitochondrial hydrogen transfer to phosphate transfer pathways, from endogenous nucleotides to mitochondrial compartmentation, from transport to mechanism, from carrier to structure, from coupling by AAC to uncoupling by UCP, and from specific to general transport laws. These wanderings are recalled with varying emphasis paid to the covered science stations. Major attention will be