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 H_2O_2, and a higher rate of apoptosis. A lack of Nnt, as in the C57BL/6J mouse, thus favours an even higher level of H_2O_2, apoptosis and counteracts tumours. The mechanisms involved will be discussed.

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PL.13

The mechanism and regulation of F-ATPases

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More than 25 high-resolution structures of mitochondrial F_1-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 F_1-ATPase inhibited with yeast inhibitor protein, IF_1, 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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SEL.1

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 Ca^{2+}. 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 Ca^{2+} concentration. In conditions of cytoplasmic Ca^{2+} overload mitochondria could thus store large amounts of precipitated Ca^{2+}-phosphate, permitting cells to overcome situations of Ca^{2+} emergency. Work in the 1960s established that the uptake of Ca^{2+} occurred electrophoretically on an unidentified carrier, and was released *via* a Na^+/Ca^{2+} antiporter (a H^+/Ca^{2+} release exchanger was also identified, and a permeability transition pore was later also found to mediate the efflux of Ca^{2+} from mitochondria). In the mitochondrial matrix two dehydrogenases, pyruvate dehydrogenase and phosphate phosphatase, were found to be regulated by Ca^{2+} The uptake process had very low affinity for Ca^{2+}: since the bulk concentration of cytosolic Ca^{2+} is in the low to mid-nM range, it was difficult to postulate a role of mitochondria in the regulation of cell Ca^{2+}. Nevertheless, energy linked Ca^{2+} 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 Ca^{2+} in the cytoplasm is not uniform: as perimitochondrial micropools of high Ca^{2+} concentration, sufficient to activate the low affinity uniporter, are created by the agonist-promoted discharge of Ca^{2+} from vicinal stores. Mitochondria thus regained center stage as important regulators of cytoplasmic Ca^{2+} (not only of their own internal Ca^{2+}). Their Ca^{2+} 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 Ca^{2+} transport is the involvement in the disease process. Ca^{2+} signaling defects are now gaining increasing importance in the pathogenesis of diseases, particularly in neurodegenerative diseases.

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SEL.2

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

paid the regulation of uncoupling proteins and to carrier transport mechanisms.

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SEL.3

Interruption of the organismal senescence program

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Mitochondria-targeted cationic plastoquinone derivatives (SkQs) operate as antioxidants in two quite different ways: (i) directly (by preventing cardiolipin peroxidation) and (ii) indirectly (by fatty acid cycling resulting in mild uncoupling which inhibits ROS formation in State 4). The quinol and cationic moieties of SkQs are involved in cases (i) and (ii), respectively. In case (i) $SkQH_2$ interrupts propagation of chain reactions involved in peroxidation of unsaturated fatty acids in cardiolipin, the formed $SkQ^{\bullet -}$ being reduced back to $SkQH_2$ by heme b_H of complex III. Molecular dynamics simulation showed that there are two stable conformations of SkQ1 with the quinol residue localized near peroxyl radicals at C_9 or C_{13} of the cardiolipin linoleate residues. In case (ii), fatty acid cycling is involved, which consists of (a) transmembrane movement of the SkQ cation/fatty acid anion pair and (b) back flows of SkQ cation and protonated fatty acid. The cycling results in H^+ conductance of planar phospholipid membranes and liposomes. In mitochondria, the cycling causes mild uncoupling, thereby decreasing membrane potential and ROS generation coupled to reverse electron transport. In yeast cells, dodecyltriphenylphosphonium (C_{12}TPP), the cationic part of SkQ1, induces uncoupling that is mitochondria-targeted since C_{12}TPP is specifically accumulated in mitochondria and increases the H^+ conductance of the mitochondrial inner membrane. The outer cell membrane conductance is not affected by C_{12}TPP. In human cell cultures, plastoquinonyl decyltriphenylphosphonium (SkQ1) and its analog, plastoquinonyl decylrhodamine 19 (SkQR1) arrest H_2O_2-induced apoptosis. When tested *in vivo*, SkQs (i) prolong lifespan of fungi, crustaceans, insects, fish, and mice, (ii) suppress appearance of many traits typical for age-related senescence (cataract, retinopathies, achromotrichia, balding, osteoporosis, decline of immune system, myeloid shift of blood cells, activation of apoptosis, induction of β-galactosidase, phosphorylation of H2AX histones, etc.), and (iii) lower tissue damage and save the lives of young animals after treatments resulting in kidney ischemia, rhabdomyolysis, heart attack, heart arrhythmia, and stroke. It is assumed that SkQs interrupt execution of programs responsible for both senescence and fast "biochemical suicide" of organism after a severe metabolic crisis.

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W.1

Towards a quantitative systems level understanding of live-cell mitochondrial physiology in health and disease

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Mitochondria are critically involved in cell cycle regulation, apoptosis, Ca^{2+} signaling, organismal development, immune responses and dynamic modulation of metabolic capacity. Mitochondrial dysfunction takes a central place in the etiology of many human disorders including diabetes, genetic oxidative phosphorylation defects, cancer and neurodegenerative disorders. At the (sub)cellular level, metabolism is linked to dynamic alterations in mitochondrial motility, position, structure, mass and function. We focus on gaining a quantitative and mechanistic understanding of the coupling between mitochondrial dynamics and function, and its regulation, at the (sub) cellular level. To this end, chemical and proteinaceous reporter molecules are introduced in living cells followed by perturbation of mitochondrial dynamics and/or function by genetic and/or chemical means. The effects of these maneuvers are studied using classical biochemical techniques, quantitative (sub)cellular (high-content) live cell microscopy, cellular and mitochondrial single-molecule spectroscopy, image processing and analysis, and quantitative deterministic/stochastic *in silico* modeling. This approach is used to obtain a systems level understanding of live-cell mitochondrial physiology by investigating: (**I**) the pathophysiology of mitochondrial dysfunction in patient cells and knockout mouse models, (**II**) the physicochemical properties of the mitochondrial matrix, (**III**) how cells can adapt to mitochondrial dysfunction at the metabolic, structural and functional level, and (**IV**) which drugs mitigate mitochondrial dysfunction at the cellular and organismal level.

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W.2

Single channel properties and modulation of intracellular chloride channels

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The work focuses on observation of the properties, functional significance, and modulation of mitochondrial chloride (mtCl) single channels using bilayer lipid membrane (BLM) method. The crude rat heart mitochondria and submitochondrial particles (inner membrane vesicles) were isolated from the hearts of male Wistar rats. The vesicles containing mtCl channels were fused into BLM and the single chloride channel currents were measured at 250/50 mmol/l KCl cis/trans solutions. Measurements of parameters such as conductance, Cl^-/K^+ selectivity, voltage or pH dependence as well as their modulation by endogenous and exogenous compounds (ATP, Mg^{2+}, H_2S) using