P/16 Uncoupling Protein 2: Physiology data and biochemistry questions

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The mitochondrial Uncoupling Protein 2 UCP2 is a member of the mitochondrial carrier family and belongs to the UCP subfamily. It is widely expressed in tissues. In immune cells, UCP2 has a regulatory function through its effect on the production of reactive oxygen species and MAPK signalling. $Ucp2^{-/-}$ mice are resistant to infection by parasites and intracellular bacteria but are more sensitive to chronic inflammation and experimental neurodegeneration. We found that autoimmune diabetes was strongly accelerated in $Ucp2^{-/-}$ mice compared to $Ucp2^{+/+}$ mice with increased intra-islet lymphocytic infiltration. These data highlight UCP2 as a new player in autoimmune diabetes. In addition, in agreement with the known inhibitory role of UCP2 on insulin secretion, loss of function of UCP2 contributes to congenital hyperinsulinism in patients. The question of whether UCP2 fully uncouples respiration from ATP synthesis is still debated. $Ucp2^{-/-}$ cells display enhanced proliferation associated with a metabolic switch from fatty acid oxidation to glucose metabolism. This metabolic switch requires the unrestricted availability of glucose, and $Ucp2^{-/-}$ cells more readily activate autophagy than wild-type cells when deprived of glucose. Altogether, these results suggest that UCP2 promotes mitochondrial fatty acid oxidation while limiting mitochondrial catabolism of pyruvate. UCP2 expression is also required for efficient oxidation of glutamine in macrophages. This role of UCP2 in glutamine metabolism appears independent from its uncoupling activity.

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P/17 Nitric oxide: Mitochondrial interactions in physiology and pathophysiology

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At physiological concentrations nitric oxide (NO) inhibits mitochondrial cytochrome *c* oxidase in competition with oxygen. Using a technique based on visible light spectroscopy we have demonstrated that endogenous NO enhances the reduction of the electron transport chain, thus enabling cells to maintain their VO_2 at low $[O_2]$. This favours the release of superoxide anion, which initiates the transcriptional activation of NF-κB as an early stress signalling response. We have recently used this technique to demonstrate that NO is inactivated by cytochrome *c* oxidase in its oxidised state and that cessation of such inactivation at low $[O_2]$ may account for hypoxic vasodilatation. Many cells respond to a decrease in oxygen availability via stabilisation of hypoxia-inducible factor-1α (HIF-1α), whose accumulation is normally prevented by the action of prolyl hydroxylases. We have found that inhibition of mitochondrial respiration by low concentrations of NO leads to inhibition of HIF-1α stabilisation. This prevents the cell from registering a state of hypoxia at low $[O_2]$, which would otherwise result in upregulation of defensive genes associated with, for example, glycolysis and angiogenesis. Furthermore, upon inhibition of mitochondrial respiration in hypoxia, oxygen is redistributed toward non-respiratory oxygen-dependent targets.

Our results demonstrate that NO acts not only as a physiological regulator of cell respiration but also as a signalling agent in the mitochondria and a controller of the distribution of available oxygen. Such mechanisms may also be involved in the initiation of pathophysiology.

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P/18 An attempt to arrest the aging program by means of mitochondria-targeted plastoquinone

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A concept is developed considering aging as an evolution-facilitating program which is unnecessary and counterproductive for human and, therefore, should be arrested. To do this, an attempt is undertaken to inactive mitochondria-produced reactive oxygen species (ROS) which seem to mediate execution of the aging program. We synthesized mitochondria-targeted, rechargeable, hydrophobic antioxidant composed of plastoquinone and cation of decyltriphenylphosphonium (SkQ1). It is shown that very low (nmol/kg/day) amounts of SkQ1 increase the lifespan of a fungus (*Podospora anserine*), invertebrates (*Ceriodaphnia affinis* and *Drosophila melanogaster*) and a mammal (mouse). Even more important, in mice SkQ1 abrogates development of such typical traits of senescence as osteoporosis (kyphosis), decrease in resistance to infections, depression, alopecia, loss of whiskers, gray hairs, disappearance of regular oestrous cycles, etc. In rats, pretreatment with SkQ1 or its homolog, SkQR1, has favourable effect in the cases of experimental heart arrhythmia, heart and kidney infarction or stroke. In rats, rabbits, cats, dogs and horses, drops of 250 nM SkQ1 prevent development of certain types of cataract, retinopathies and uveitis and in some cases return vision to animals that became blind due to these pathologies. These data are consistent with the assumption that SkQ1 interferes with execution of aging program and is promising in treating some age-related diseases.

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P/19 Evidence for the presence of a peroxide in the binuclear site of oxidized cytochrome *c* oxidase: The new catalytic cycle

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Cytochrome *c* oxidases, members of the superfamily of heme-copper containing terminal oxidases, catalyse the reduction of molecular oxygen to water. Catalytic cycles consist of the oxidized O-state, the one and two electron reduced E and R-states. The R-state reacts with oxygen to form the P-state, input of the third electron leads to the F-state, which is converted to the O-state again after receiving the fourth electron. The P- ("Peroxy") and F- (Ferryl) states are oxoferryl states. Presently the binuclear heme Fe_{a3}–Cu_B active site is believed to contain either a hydroxyl group

at Cu_B in the O-state, or an additional hydroxyl group at Fe_{a3}. However, the electron densities of all high resolution structures are compatible best with a peroxo-bridge between Fe_{a3} and Cu_B. In addition, we find that the F-state generated in the traditional way by an excess of H_2O_2, can be converted into a P-state simply by addition of catalase. Finally we have discovered conditions under which the O-state spontaneously converts into a P-state with concomitant formation of a tyrosine radical. All these results can be understood best if the catalytic cycle starts with an O-state containing a bridging peroxide dianion and the F-state contains a superoxide bound to Cu_B.

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P/20 The proton translocation mechanism of cytochrome *c* oxidase

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The cytochrome *c* oxidases (CcO), which are responsible for most of the O_2 consumption in biology, are also redox-linked proton pumps that effectively convert the free energy of O_2 reduction to an electrochemical proton gradient across mitochondrial and bacterial membranes. Recently, time-resolved measurements have elucidated the sequence of events in proton translocation, and shed light on the underlying molecular mechanisms. One crucial property of the proton pump mechanism has received less attention, *viz*. how proton leaks are avoided. Here, we will analyse this topic and demonstrate how the key proton-carrying residue Glu-242 (numbering according to the sequence of subunit I of bovine heart CcO) functions as a valve that has the effect of minimising back-leakage of the pumped proton.

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P/21 The proton pumping heme-copper oxidases

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All known proton pumping respiratory oxidases are members of the heme-copper superfamily. This superfamily contains not only the oxygen reductases (respiratory oxidases) but also prokaryotic NO reductases that are used for denitrification and detoxification. Our work has focused on the largest group of respiratory oxidases, the A-family, with the aim being to understand how the chemistry of reducing oxygen to water is coupled to driving a unidirectional proton pump. Most studies have used the aa_3-type oxidase from *R. sphaeroides*. Mutations in one of the two proton input channels, the D channel, can decouple the proton pump from the redox chemistry. The properties of these mutants will be discussed. Recent studies have included respiratory oxidases that are not members of the major (canonical) family of heme-copper oxidases, but are in the B- and C-families. This includes work on the ba_3-type oxidase from *Thermus thermophilus*, in a collaboration with the group of Dr. James Fee (Scripps Institute). Results will be discussed.

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P/22 Cytochrome *c* binding to the cytochrome bc_1 complex: An interaction critical for electron transfer

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In cellular respiration, the mobile electron carrier cytochrome *c* shuttles electrons from cytochrome bc_1 complex to cytochrome *c* oxidase. The X-ray structure of the complex between cytochrome *c* and cytochrome bc_1 complex at 2.97-Å resolution gave the first structural insight for such a complex from the respiratory chain. The structure revealed the general features of the interface, which is well suited for transient interaction and fast turnover. Remarkably, cytochrome *c* binds to only one recognition site of the homodimeric complex. We now determined the structure of the electron transfer complex in the reduced state at 1.9-Å resolution. The high resolution allows an accurate description of the interface, especially of electrostatic and water-mediated interactions. The dimer structure is asymmetric. Monovalent cytochrome *c* binding is correlated with conformational changes of the Rieske head domain and subunit QCR6p and with a higher number of interfacial water molecules bound to cytochrome c_1. Comparison with a second structure obtained for isoform-2 cytochrome *c* bound to the cytochrome bc_1 complex led to the definition of a minimal interface, the so-called core interface, which is present in all of these structures. The importance of single core interface residues for formation of the reactive complex in solution was probed by site-directed mutagenesis and characterization of the variants.

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P/23 Toward a mitochondrial therapy of collagen VI muscular dystrophies

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Ullrich Congenital Muscular Dystrophy (UCMD) and Bethlem Myopathy (BM) are muscle diseases due to mutations in the genes encoding the extracellular matrix protein collagen VI. Generation of a dystrophic mouse model where collagen VI synthesis was prevented by genetic ablation of the *Col6a1* gene allowed an investigation of pathogenesis, which revealed the existence of a Ca^{2+}-mediated dysfunction of mitochondria and the sarcoplasmic reticulum. A key event appears to be inappropriate opening of the mitochondrial permeability transition pore, an inner membrane high-conductance channel. Consistently, the *Col6a1*$^{-/-}$ myopathic mice could be cured with cyclosporin A through inhibition of cyclophilin D, a matrix protein that sensitizes the pore to opening. Studies of myoblasts from UCMD and BM patients demonstrated the existence of a latent mitochondrial dysfunction irrespective of the genetic lesion responsible for the lack or the alteration of collagen VI. These studies suggest that PTP opening may represent the final common pathway for skeletal muscle fiber death; and provided a rationale for a pilot clinical trial with cyclosporin A in patients affected by UCMD and BM. Prior to treatment, all patients displayed mitochondrial dysfunction and increased frequency of apoptosis, as determined in muscle biopsies. Both these pathological signs were largely normalized after 1 month of oral cyclosporin A administration, which also increased muscle regeneration. These results indicate that mitochondrial dysfunction