

Session 4: Signalling To and From the Mitochondria – II.



4-01. Reconstitution of kinase signaling in mitochondria - how mitoK_{ATP} opening inhibits permeability transition opening.

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Pharmacological preconditioning can be triggered by an intracellular signaling pathway in which G_i-coupled surface receptors activate a cascade including PI3K, eNOS, guanylyl cyclase and protein kinase G (PKG) [1]. Activated PKG opens the mitochondrial K_{ATP} channel (mitoK_{ATP}) and mitoK_{ATP} opening causes increased production of reactive oxygen species (ROS) [2,3], which then go on to activate other kinases. The steps between PKG and mitoK_{ATP} opening are unknown, as are the steps downstream of mitoK_{ATP}.

We found that exogenous PKG + cGMP induces mitoK_{ATP} opening in isolated heart mitochondria to the same extent as K_{ATP} channel openers such as diazoxide or cromakalim. This effect was blocked by mitoK_{ATP} blockers — 5-HD, glibenclamide, and TPP⁺, by the PKG-selective inhibitor KT5823, and by protein kinase C (PKC) inhibitors chelerythrine, Ro318220, and the highly selective PKC-peptide antagonist, V₁₋₂. We also found that mitoK_{ATP} is opened by the PKC activators 12-phorbol 13-myristate acetate and H₂O₂. We conclude that PKG is the terminal cytosolic component of the signaling pathway and that it transmits the cardioprotective signal from cytosol to inner mitochondrial membrane by a pathway that includes PKC-.

K_{ATP} channel openers or activators of PKG or PKC inhibited MPT opening, and this effect was also mediated by a PKC-. Inhibition of MPT opening was prevented by MPG, indicating that the signal was transmitted by a mitoK_{ATP}-dependent increase in H₂O₂.

The effect of PKG + cGMP requires an intact outer membrane, whereas the effects of the two PKC-s do not. Indeed both effects of PKC- activation — mitoK_{ATP} opening and MPT inhibition — were observed in mitoplasts, implying that these PKCs are tightly bound to the inner membrane. This partial resolution of the mitochondrial portion of the cardioprotective signaling pathway should enable us to identify the kinase that phosphorylates mitoK_{ATP}.

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4-02. Modulation of energy transfer between mitochondria and myofibrils by changes of cardiac work.

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In the heart, the energy supplied by the mitochondria to the myofibrils is continuously adjusted to the contraction requirement for a wide range of cardiac loads. This process, finely tuned over a broad range of mechanical requirement, is mediated by

a complex specialized enzyme system. The contribution of creatine kinase (CK) shuttle in transferring the energy between mitochondria and myofibrils may vary with the changes in cardiac work. For example, we have observed that the export of ATP from mitochondria to myofibrils is bypassing the CK shuttle if ATP synthesis is partially inhibited [1,2].

The aim of this study was to determine the dependence of the energy transfer pathways between mitochondria and myofibrils on cardiac workload. We studied the energy transfer pathways by analyzing the ^{31}P -NMR magnetization transfer data using Bloch-McConnell equations [3]. The data was acquired on isovolumetric Langendorff perfused hearts. The work was varied by changing the external calcium concentration $[\text{Ca}]$ (from 0.5 mM to 4.0 mM) and/or by beta adrenergic stimulation. In our analysis, we computed the share of energy transfer through different pathways, consistent with the observed magnetization changes. For this, mathematical models were composed taking into account the forward and reverse CK reactions, ATPase activity as well ATP synthesis in mitochondria. Several possible energy transfer pathways were considered with different levels of intracellular compartmentation. The simplest model considered in our study was the non-compartmentalized model exchanging magnetization between PCr, ATP, and Pi (three-site model). The most complex one consisted of three compartments for ATP (mitochondrial matrix, cytoplasm, and myofibrillar compartment), three isoforms of CK, ATPase and ATP synthase connecting compartmentalized ATP with PCr and Pi.

At low work, ($[\text{Ca}]=0.5$ mM), all considered energy transfer schemes were able to reproduce the measured magnetization transfer within the experimental errors. This includes the simplest considered scheme (the three-site model) as well. However, when the beta adrenergic stimulation was used with high $[\text{Ca}]=4.0$ mM, the simple non-compartmentalized model was not able to fit the data: ATP compartmentalization must be taken into account. When compartmentalized models were used, it was possible to separate the fluxes through two isoforms of CK - the mitochondrial and the cytoplasmic one. According to the experimental data, the total forward rate of CK reaction is almost constant if the cardiac work is changed by increasing $[\text{Ca}]$ from 0.5 mM to 4.0 mM. However, the forward and the backward rates of subcellular CK isoenzymes are changing with cardiac work. Namely, a rise in cardiac work increased both mitochondrial PCr production in mitochondria and myofibrillar PCr utilization at constant global CK flux.

In addition, the compartmentalized models allow one to find the fraction of ATP which is transported as PCr by the CK shuttle and directly as ATP from mitochondrial matrix to myofibrils. Our analysis of the data suggests that both pathways (direct export of ATP from the matrix to myofibrils and the CK shuttle) are probably used, but depending on the work their proportion might vary. In extreme conditions of a work demand exceeding mitochondrial ATP synthesis capacity (beta stimulation and high calcium): global CK flux decrease and ATP is mainly exported directly.

These findings suggest that the CK shuttle is able to support the energy transfer, except in extreme conditions. This may have implications in understanding the process of cardiac pathology.

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4-03. Comparison of the effects of nonesterified fatty acids and of their ethanolamine amides (*N*-acylethanolamines) on isolated mitochondria and on mitochondria within intact cells, including triggering of apoptosis.

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Nonesterified long-chain fatty acids are excellent energy-providing respiratory substrates for numerous tissues but they are also well-known protonophores and uncouplers of oxidative phosphorylation for isolated mitochondria and mitochondria within intact cells [1]. Elevated concentrations of fatty acids can elicit cell death, both necrotic and apoptotic [2]. *N*-Acylethanolamines (NAEs) form a class of important fatty acid derivatives that have recently attracted attention because of their formation and accumulation in injured heart and brain [3]. The main representative of these compounds, *N*-arachidonylethanolamine (also called anandamide) may have signalling functions and also acts as ligand for cannabinoid receptors. The present work compares some effects of nonesterified fatty acids with those of NAEs.

In isolated rat heart mitochondria NAEs were much weaker protonophores than corresponding nonesterified fatty acids [4]. In contrast, in the presence of micromolar concentrations of Ca^{2+} , long-chain unsaturated NAEs, *N*-arachidonylethanolamine and *N*-oleylethanolamine, were potent openers of the mitochondrial permeability transition pore. They also acted as weak inhibitors of mitochondrial respiration, in particular of complex I of the respiratory chain. As weak uncouplers, NAEs decreased the rate of the formation of reactive oxygen species (ROS) by respiring mitochondria. However, *N*-arachidonylethanolamine partly prevented the drastic decrease of ROS formation produced by chemical uncouplers. In this respect, it exerted a similar, though much weaker, effect as that by known inhibitors of complexes I and III, rotenone and antimycin A.

Acting on cells in culture, arachidonic acid and, to a lesser extent, oleic acid elicited apoptotic cell death in the following cell types: rat hepatoma AS-30D, mouse Ehrlich ascites carcinoma, human lymphoblastoid Jurkat cells and human leukemia HL-60 cells. However, mouse neuroblastoma N2a cells appeared resistant to arachidonic and oleic acids. Interestingly, this cell line was also more resistant to ultraviolet irradiation than the former cell lines. Apoptosis induced in susceptible cell lines by arachidonic and oleic acids proceeded along the mitochondrial pathway characterized by release of cytochrome c from mitochondria to the cytosol and activation of caspase-3.

Conflicting information exists in the literature on proapoptotic and antiproliferating activity of *N*-arachidonylethanolamine. In the present study we were unable to induce apoptosis by NAEs in rat myoblasts H9c2, primary rat neonatal cardiomyocytes and mouse neuroblastoma N2a cells. Further studies in this line are, however, required.

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4-04. Mitochondrial GTP metabolism - a main function of NDP-kinase D?

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Isoenzymes of hexameric nucleoside diphosphate kinase (NDPK/nm23) function in NTP-biosynthesis ($\text{NDP} + \text{ATP} \leftrightarrow \text{NTP} + \text{ADP}$) and have additional, multi-faceted roles in cell signaling, proliferation and differentiation [1]. Detailed subfractionation of rat liver cells and surface plasmon resonance spectroscopy [2] with recombinant protein revealed that the NDPK-D isoenzyme [3] has a unique intramitochondrial localization at the inner mitochondrial membrane, where it firmly binds to acidic phospholipids, mainly cardiolipin. The latter high affinity interaction (K_D about 30 nM) is due to the NDPK-D-specific arginine 90 in a basic RRK motif at the surface of the NDPK hexamer, since a R90D mutation abolishes cardiolipin interaction. Latency assays with liver, HEK and HeLa mitochondria suggest that most NDPK-D is oriented towards the intermembrane and cristae space, while a variable fraction may be oriented towards the matrix space. The physiological role of NDPK-D was analyzed with HeLa cell lines that can express NDPK-D under the control of an inducible tetracycline (tet) promoter. Mitochondrial respiration from control cells was only weakly stimulated with NDPK substrate TDP, while it was strongly stimulated in NDPK-D overexpressing tet-treated cells, together with a decrease in K_m (ADP). Thus, NDPK-D uses NDP nucleotides to locally regenerate ADP in the mitochondrial intermembrane space, which in turn stimulates oxidative phosphorylation. From the NTP generated, mainly GTP could be important for GTP-dependent processes in the intermembrane compartment, like e.g. GTP-binding proteins involved in mitochondrial dynamics.

We propose a model for NDPK-D participating in GTP-export from the matrix space and GTP-regeneration in the intermembrane space by association of the NDP kinase hexamer with adenylate translocator of the inner membrane via cardiolipin patches, similar to proteolipid complexes formed by octameric mitochondrial creatine kinase [4].

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4-05. Inter-genomic cross talk between the mitochondria and nucleus in aging and cancer.

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As an important part of 'free-radical theory of aging' mitochondria play a fundamental role both as producers and targets of ROS. Mitochondrial DNA (mtDNA) is extremely susceptible to ROS damage compared to genomic DNA because of ROS production by the mitochondria, the absence of protective histones and limited DNA repair. Thus individuals accumulate mtDNA mutations as they age which leads to compromised mitochondrial function.

To understand the biological consequences of mtDNA mutation accumulated during aging we have created mitochondrial gene knock out cell lines (ρ^0). Our study suggests

that ρ^0 cells demonstrate characteristic features of cellular aging. Mitochondrial gene knock out ρ^0 cells showed typical morphology associated with aging such as increased size and elongated appearance. They have increased senescence-associated β -Gal activity, lipofuscin pigment and plasminogen activator inhibitor-1 expression. Consistent with their decreased proliferation, the expression of mitotic cyclins was decreased and that of cdk inhibitors was increased. Retinoblastoma (Rb) hypophosphorylation and decreased telomerase activity were also noted. Using this cellular model and cybrid cell technology, we provide evidence that (1) inactivation of mitochondrial genes leads to chromosomal instability (CIN) that are present in a variety of human tumors and (2) mitochondrial gene knockout cells show transformed phenotype. Our study also demonstrates that mitochondrial genetic status plays a key role in regulation of a multifunctional protein APE1 (also known as Ref1 or HAP1) involved in transcription and DNA repair in the nucleus and the mitochondria. Our study revealed that altered expression of APE1 in ρ^0 cells and tumorigenic phenotype can be reversed by exogenous transfer of wild type mitochondria in ρ^0 cells. Furthermore, we demonstrate that APE1 expression is altered in variety of primary tumors. Taken together, these studies suggest that inter-genomic cross talk between mitochondria and the nucleus plays an important role in tumorigenesis and that APE1 mediates this process. Our study support the mitochondrial theory of aging and suggest that ρ^0 cells can serve as an *in vitro* model for cellular aging.

4-06. A toxic halogenated cysteine S-conjugate has deleterious effects on mitochondrial physiology.

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Many toxicants impair mitochondrial physiology leading to a decrease in energy production. Such a malfunction could lead to progressive organ failure. Humans are continuously exposed to reactive electrophiles derived from the environment, medications and food, and as natural metabolites within the body. Many are detoxified through acetylation of the corresponding cysteine S-conjugate to the mercapturate, which is excreted. However, if the cysteine S-conjugate contains a good leaving group at the sulfur, a β -elimination reaction may compete with the acetylation reaction. Enzymes that catalyze this elimination reaction are known as cysteine S-conjugate β -lyases. These enzymes convert susceptible cysteine S-conjugates to pyruvate, ammonium and a sulfur-containing fragment (RSH). If the eliminated fragment is not particularly reactive, the parent cysteine S-conjugate is not generally toxic. On the other hand, if RSH is reactive, the parent cysteine S-conjugate is potentially toxic. Halogenated alkenes, such as tetrafluoroethylene, are examples of environmental and workplace contaminants that are toxified (bioactivated) at least in part by the action of cysteine S-conjugate β -lyase(s) on the corresponding cysteine S-conjugate. The cysteine S-conjugate derived from tetrafluoroethylene [(1,1,2,2-tetrafluoroethyl)-L-cysteine (TFEC)] is converted by cysteine S-conjugate β -lyases to an RSH fragment that is a thioacylating agent, particularly of protein lysine residues. TFEC (and other halogenated cysteine S-conjugates) are mitochondrial toxicants, presumably as a result of the presence of cysteine S-conjugate β -lyase activity in these organelles [1,2]. A major cysteine S-conjugate β -lyase in mitochondria is mitochondrial aspartate aminotransferase (mitAspAT) [3]. In the presence of TFEC the enzyme is inactivated on average after several thousand turnover events [3]. Exposure of rat kidney *in vivo* and PC12 cells in culture to TFEC results in selective inhibition of mitochondrial enzymes of energy metabolism, including mitAspAT, aconitase, and the E2 and E3 components of the α -ketoglutarate dehydrogenase complex (KGDHC) [reviewed in 4].

In the present work, isolated rat liver mitochondria energized with succinate were incubated in the presence or absence of TFEC. Four physiologically important mitochondrial parameters (O_2 uptake, Ca^{2+} flux, mitochondrial membrane potential ($\Delta\psi_m$), and swelling) were simultaneously measured using a uniquely designed multiparameter chamber. A concentration- and time-dependent disruption of these parameters by TFEC was observed: (1) inhibition of mitochondrial respiration, (2) Ca^{2+} release from mitochondrial matrix, and (3) dissipation of $\Delta\psi_m$. Both the lag-period and the degree of maximal swelling of mitochondria induced by TFEC were decreased with increasing concentration and time of pre-incubation. KGDHC and mitAspAT were both inhibited by ~20 % in liver mitochondria exposed to TFEC. No change was found in the activities of glutamate- or malate dehydrogenases.

Possible exposure to endogenous and exogenous electrophiles (that can be bioactivated via the cysteine *S*-conjugate β -lyase pathway) may lead to selective loss of enzymes involved in energy metabolism. This loss may contribute to (or exacerbate) the mitochondrial dysfunction associated with aging and many neurodegenerative diseases.

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4-07. ν -Raf antagonizes impairment of mitochondrial respiratory function following growth factor removal.

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The cell's ability to respond to extrinsic stimuli depends on the provision of adequate energy. Increasing evidence suggests that cellular energy production itself is subject to regulation by extrinsic signals through signaling pathways, which control cell proliferation and survival. In tumors, where signaling components are frequently affected by mutations, an increased dependence on glycolytic energy has been recognized long ago and more recent experiments suggested direct targeting of components of the glycolytic machinery as one of the underlying mechanisms [1,2]. Indirect evidence from studies on the control of cell survival by C-Raf suggests a role for this kinase in maintaining mitochondrial integrity during apoptosis induction [3]. These experiments also suggested cooperation in this process with two other major guardians of cell survival, Bcl-2 and PKB. To test for direct effects of C-Raf on mitochondrial energy production we performed high resolution respirometry on the mouse pro-myeloid 32D cell line. These cells strictly depend on IL-3 for growth and survival. IL-3 removal results in growth arrest and subsequent apoptosis, which can be prevented through over-expression of the oncogenic form of C-Raf, ν -Raf. In the comparison of the effects of growth factor withdrawal on mitochondrial respiratory function in 32D cells *versus* 32D cells protected by ν -Raf, our experimental design focused on early time points before cells become irreversibly committed to cell death.

Cells were incubated in RPMI 1640 + 10% FCS supplemented with penicillin-streptomycin and 2 mM L-glutamine without IL-3 for a period of 8 h at a cell density of $0.5 \cdot 10^6$ cells·ml⁻¹. Controls were cultured in the same medium supplemented with IL-3 (15 % WEHI). During the 8 h time interval, no significant difference in viability (<4 % trypan blue or annexin V staining) was observed between control and growth factor deprived cells. The respiratory activities of intact cells were measured using the OROBOROS Oxygraph-2k for high resolution respirometry. After recording cellular routine

respiration (C_r) in the respective incubation media, ATP-synthase was inhibited by oligomycin ($C_{r,o}$), followed by a stepwise FCCP titration to achieve maximum uncoupled respiration in intact cells ($C_{r,u}$). Respiration was then inhibited by rotenone and antimycin A. Data (means \pm SD) were analysed by a paired t-test.

Activities of citrate synthase (mitochondrial matrix marker enzyme) and lactate dehydrogenase (glycolytic marker enzyme) per million cells remained unchanged, irrespective of IL-3 withdrawal, indicating that mitochondrial content and glycolytic capacity were maintained. Analysis of ERK and AKT phosphorylation, two main signaling effectors of the IL-3 receptor, revealed no measurable decline in their activities at the end of the WEHI starvation period. However, a significant decrease in cell volume (measured by CASY[®]) was observed in 32D (0.93 ± 0.10 pL versus 0.76 ± 0.05 pL) but not in cells protected by v-Raf. Regardless of the significant decrease in cell size, the protein content of 32D cells remained unaffected upon IL-3 withdrawal.

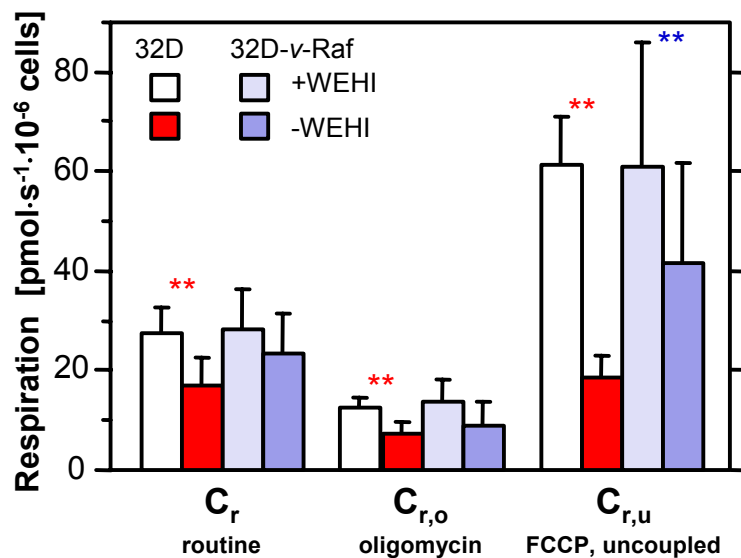
Mitochondrial respiratory function of IL-3-deprived 32D cells dropped significantly in all respiratory states (Fig. 1). Routine and oligomycin-inhibited respiration of 32D-v-Raf cells deprived of IL-3 were not significantly lower compared to their controls with IL-3 (Fig. 1).

The uncoupling control ratio ($UCR = C_{r,u}/C_r$) is a sensitive indicator for the integrity of mitochondrial function in intact cells. In 32D controls, the UCR declined from 2.3 ± 0.23 to 1.23 ± 0.61 after growth factor removal. In contrast, the UCR of 32D-v-Raf cells remained unaffected by IL-3 withdrawal. The inverse of the respiratory control ratio ($C_{r,o}/C_{r,u}$), an index of the extent of oligomycin inhibited leak rate of respiration relative to the maximum capacity of the respiratory chain, was significantly different between controls (0.20 ± 0.01) and growth factor deprived 32D cells (0.43 ± 0.18), indicating primarily the loss of respiratory capacity, and providing indirect evidence for simultaneous partial uncoupling [4].

Even though IL-3 withdrawal showed a significant effect on the respiratory rates of the different respiratory states of 32D cells, the oxygen kinetics in coupled intact cells was not significantly affected, with p_{50} values of 0.044 ± 0.001 kPa for controls and 0.039 ± 0.006 kPa for IL-3 deprived cells.

The decline of mitochondrial respiratory capacity comprised an early event in the pathway to apoptosis after growth factor withdrawal, before the onset of inactivation of the main signaling effectors of the IL-3 receptor. This time course suggests a primary role of mitochondrial respiratory function in these cells. Our results clearly demonstrate that IL-3 withdrawal severely compromises mitochondrial respiratory function in a fashion that is almost completely suppressible by v-Raf. This for the first time suggests a direct link between the key mitogenic and survival kinase C-Raf and mitochondrial energy homeostasis.

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4-08. Modulation of mitochondrial respiratory parameters by the human papillomavirus type 16 E7 oncoprotein.

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Changes in cellular carbohydrate metabolism are a hallmark of malignant transformation and represent one of the earliest discernible events in tumorigenesis. We previously showed that the E7 oncoprotein targets the glycolytic key regulator pyruvate kinase subtype M2 (M2PK) and this leads to downregulation of M2PK activity and changes in the glycolytic flux [1]. There is evidence that different oncogenes, such as ras and HPV 16 E7, cooperate in a complex way to establish the metabolic phenotype of tumor cells which supports the proliferative state [2,3].

We analyzed, whether mitochondrial oxidative phosphorylation, representing another important energy producing system, is influenced by the HPV 16 E7 oncogene. We used a cell line derived from normal rat kidney (NRK) cells, called 14/2 cells, which contain a hormone inducible expression vector for HPV-16 E7 [4]. All experiments were performed after 4 h of dexamethasone-induced E7 expression [2]. Mitochondrial respiratory function was analyzed by high-resolution respirometry with the OROBOROS® Oxygraph-2k. The experimental regime started with routine respiration, followed by inhibition of ATP synthase with oligomycin, and uncoupling by stepwise titration of FCCP. Finally, respiration was inhibited by sequential addition of rotenone and antimycin A [5].

Cellular routine respiration was decreased after 4 h induction of the E7 protein, whereas oligomycin-inhibited and uncoupled respiration remained unchanged. This resulted in an increased uncoupling control ratio, and a decreased phosphorylation respiratory control ratio (RCRp [5]). The activity of the mitochondrial matrix marker enzyme citrate synthase was comparable between induced and uninduced cells, which confirmed the results obtained when relating respiratory parameters per cell number. Taken together, these data suggest that the observed phenomenon might be linked to modulations of cellular metabolism by the human papillomavirus. However, the exact mechanisms how an oncoprotein can influence the activity of the respiratory chain remain to be elucidated.

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4-09. Keratinocyte-specific knockout of the Tfam protein allows to elucidate the role of the mitochondrial respiratory chain for cell proliferation *in vivo*.

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The Tfam protein controls the amount of mitochondrial DNA (mtDNA) in the cell [1-3]. Without mtDNA, the mitochondrial respiratory chain is not functional, because mtDNA encodes 13 of its ca. 80 polypeptide subunits. A HeLa cell line without mtDNA ($\rho 0$) shows a profound proliferation defect, an effect which is however not simply due to energetic or biosynthetic problems, since levels of ATP, UTP, heme containing proteins and FeS cluster enzymes were normal. These results point to a hitherto unknown role of the mitochondrial respiratory chain in cell proliferation. To elucidate this role *in vivo*, we have bred mice with a keratinocyte-specific deletion of the Tfam protein by crossing mice having exons 6 and 7 of the TFAM gene flanked by loxP-sites (2) with mice carrying the Cre-recombinase transgene under control of the keratinocyte-specific human K14 promoter. At the day of birth, Tfam protein is still detectable, but older knockout animals show a progressive loss confined to the epidermis. At day 6 after birth, the mtDNA-encoded subunit II of cytochrome oxidase is not detectable any more. The epidermis gradually gets thinner, its epidermal stem cell compartment, the basal layer, is disordered and hair follicles fail to develop normally. The knockout mice stop gaining weight at day 3 and die between day 4 and day 7. The tongue epithelium is also disordered and papillae show a progressive degradation from day 0 to day 6. The animals develop an ulceration at the back of the tongue, the resulting pain probably inhibiting food intake, leading to the observed weight loss and ultimately death. Considering the proposal by some authors that the epidermis is a physiologically anaerobic tissue [4], the drastic effect of ablating respiratory chain activity in keratinocytes is even more striking. These results show that an intact mitochondrial respiratory chain is essential for cellular proliferation *in vitro* and proliferation and differentiation *in vitro* and *in vivo*.

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4-10. Mitochondrial hyperpolarization: a checkpoint of T-cell life, death, and autoimmunity.

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Activation, proliferation, and cell death pathway selection of T lymphocytes depend on reactive oxygen intermediates (ROI) production and ATP synthesis which are tightly regulated via the mitochondrial transmembrane potential ($\Delta\psi_m$). Mitochondrial hyperpolarization (MHP) and ATP depletion represent early and reversible steps in T cell activation and apoptosis. By contrast, T lymphocytes of systemic lupus erythematosus (SLE) patients exhibit persistent MHP, cytoplasmic alkalinization, increased ROI production, and ATP depletion that mediate enhanced spontaneous and diminished activation-induced apoptosis and sensitize lupus T cells to necrosis. Necrotic, but not

apoptotic, cell lysates activate dendritic cells and may account for increased interferon- α production and inflammation in lupus patients. MHP is proposed as a key mechanism of pathogenesis and target for pharmacological intervention in SLE. Persistent MHP was associated with increased mitochondrial mass and increased mitochondrial and cytoplasmic Ca^{2+} content in T cells and enhanced NO production by monocytes of lupus patients. Activation of T cells through the T cell receptor initiates a biphasic elevation in cytosolic free Ca^{2+} concentration, a rapid initial peak observed within minutes and a plateau phase lasting up to 48 h. In response to CD3/CD28 costimulation, rapid Ca^{2+} fluxing was enhanced while the plateau phase was diminished in lupus T cells. NO-induced mitochondrial biogenesis in normal T cells enhanced the rapid phase and reduced the plateau of Ca^{2+} influx upon CD3/CD28 costimulation, thus mimicking the Ca^{2+} signaling profile of lupus T cells. Mitochondria constitute major Ca^{2+} stores and persistent MHP and NO-dependent mitochondrial biogenesis may account for altered Ca^{2+} handling by lupus T cells. Coordinated changes in expression of genes encoding members of the electron transport chain (ETC) and anti-oxidant defenses underlie mitochondrial dysfunction and dominate the altered gene expression profile of lupus lymphocytes. Members of the ETC may serve as novel target for pharmacological intervention in SLE.

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