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Summary

"Microorganisms and granula are at an equivalent level and represent elementary organisms, which are found wherever living forces are acting, thus we want to describe them by the common term bioblasts. In the bioblast, that morphological unit of living matter appears to be found" [1].

The endosymbiotic theories link the mitochondria and plastids to their free-living ancestors. Together, these are the *bioblasts* in spotlight of bioenergetics. Bioblasts and interactions with their hosts are the topics of *Bioenergetics Communications*.

The 22nd EBEC (European Bioenergetics Conference, 2024-Aug-26 to 31), hosted in Innsbruck, Austria, presents 415 Abstracts [2,3], 33 of which are presented here as 'late-braking abstracts'. An innovative publishing format [4] is required to adapt to current trends of late submissions of abstracts (not peer reviewed) and nevertheless with secured priority of information by a collective DOI.

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[2] Gnaiger E ed (2024) EBEC2024 – What is Life? Spotlights on Mito and Chlora. Biochim Biophys Acta – Bioenergetics (in prep). https://www.sciencedirect.com/special-issue/10MRQ178S2C

[3] Tindle-Solomon L, Schmitt S, Gnaiger C, Cardoso LHD, Gnaiger E, eds (2024) EBEC2024 Abstract Book. Biochim Biophys Acta – Bioenergetics (in prep). https://www.bioblast.at/index.php/EBEC2024_Abstract_Book_2024_Biochim_Biophys_Acta_Bioenerg

[4] Gnaiger E (2021) Beyond counting papers – a mission and vision for scientific publication. Bioenerg Commun 2021.5. https://doi.org/10.26124/bec:2021-0005

Effect of pharmacological intervention – do changes in proteom reflect on alterations in bioenergetic parameters in fibroblasts from patients with Neurodegeneration with Brain Iron Accumulation (NBIA)?

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Neurodegeneration with Brain Iron Accumulation (NBIA) is a rare disease (1–3 patients / 1000000 people) expressed by an excessive iron accumulation in the brain. NBIA is usually associated with slowly progressive pyramidal and extrapyramidal symptoms, axonal motor neuropathy, optic nerve atrophy, cognitive impairment and neuropsychiatric disorders. The most common from eleven NBIA subtypes include pantothenate kinase-associated neurodegeneration (PKAN), PLA2G6-associated neurodegeneration (PLAN), mitochondrial membrane protein-associated neurodegeneration (MPAN) and 35 beta-propeller protein-associated neurodegeneration (BPAN). Interestingly, MPAN is a dominant subtype among Polish population and is associated with the mutation in *C19orf12* gene. The mechanism of how the loss of protein's function results in the disease development remines unclear, hence, there are no pharmacological therapies to date. However, few pharmacological approaches are recently investigated with the hope to attenuate symptoms of the disease.

The goal of our study is to examine whether the intervention based on influencing of metabolic pathways could have a positive effect on mitochondrial bioenergetic and cell fate of fibroblasts of MPAN patients. Our experimental approach covers both, basal and OXPHOS promoting conditions, in order to better visualize mitochondrial metabolic defect in MPAN fibroblasts.

We want to clarify whether changes observed in a cellular proteome are accompanied by the alterations in functional parameters such as: metabolic (dehydrogenase) activity, glycolysis, activity of Krebs cycle enzymes, as well as mitochondrial respiratory chain activity (oxygen consumption). This could potentially explain a beneficial effect of the pharmacological approach used in our study.

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Bioenergetic implications in a cellular model depicting the progression from steatosis to steatosis with inflammation

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According to *World Health Organization*, 60 % of adults are overweight or obese. As an adaptation to excessive food intake, hepatocytes accumulate triacylglycerols in a safe lipid droplet form and enhance fatty acid oxidation. When cells' capacity to safely store and remove excessive lipids is exceeded, metabolic dysfunction-associated steatotic liver disease (MASLD) can be developed. MASLD has been estimated to affect roughly 30% of the global population. In around 20% of these patients, the disease progresses to a more severe condition – metabolic dysfunction-associated steatohepatitis (MASH).

The aim of this study was to investigate the impact of progression from steatosis to steatosis with inflammation on bioenergetic parameters in HepG2/C3A cells. A cellular model mimicking the progressive steatotic condition in hepatocytes of MASLD patients is based on HepG2/C3A cells incubated with a mixture of free fatty acids (FFAs), reflecting the lipid composition in hepatocytes of patients with fatty liver, and FFAs in combination with tumor necrosis factor alpha (TNFα), mimicking steatosis with inflammation in MASH patients' liver. The investigated parameters included ATP level, general metabolic activity (dehydrogenases activity), glycolysis pathway activity (along with lactate levels and lactate dehydrogenase level), as well as mitochondrial oxygen consumption rate (along with the level of mitochondrial respiratory chain complexes). The obtained results provide valuable information on the impact of progression from simple steatosis to steatosis with inflammation on cellular bioenergetic parameters. This, in turn, represents important insight into the mechanism of MASLD progression, which remains poorly understood.

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Regulation of CO_2 acquisition by diatoms: study of an atypical carbonic anhydrase

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The diatom *Phaeodactylum tricornutum* is an aquatic microalga that lives in brackish and marine water[1] and exhibits CO_2 concentration mechanism (CCM) to ensures an adequate supply of CO_2 for Calvin Benson Bassham cycle (CBB). Biophysical CCM relies on the transport of HCO_3^- across the different cell compartments and the catalysis of the reversible conversion of HCO_3^- to CO_2 by carbonic anhydrase. These carbonic anhydrases are divided into eight subclasses[2] . Among these subclasses is the Iota-Carbonic anhydrase (ιCA), a distinctive enzyme that is not a metalloenzyme[3] and tends to be overexpressed in low abundance of CO_2 or zinc. This latter phenotype is surprising since most carbonic anhydrases use zinc as a cofactor[4][5]. We are aiming to investigate the function, regulation, and interaction of ιCA with other enzymes of CCM by comparing the behavior of ιCA-deletion mutant strains to wild type in low CO_2 and zinc concentrations conditions. We also aim to characterize the mode of action by purifying the enzyme, characterizing its structural state and measuring its activity. This study will allow us to unravel the photosynthetic key elements in diatoms.

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[5] Lionetto MG et al (2016) The complex relationship between metals and carbonic anhydrase: new insights and perspectives. Int J Mol Sci 17:12. https://doi.org/10.3390/ijms17010127

Cite: Abdallah O, Avilan L, Parsiegla G, Receveur Brechot V, Gontero B, Launay H (2024) Regulation of CO_2 acquisition by diatoms: study of an atypical carbonic anhydrase. Bioenerg Commun 2024.6:4. https://doi:10.26124/bec.2024-0006

A computational study to investigate the pathogenicity of single or combinations of respiratory Complex I variants

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Pathogenic variants affecting genes of respiratory complex I (CI) subunits show a variable phenotypic expression, that ranges from severe and lethal infantile encephalopathy (Leigh syndrome) to adult-onset milder phenotypes, as the Leber hereditary optic neuropathy (LHON). Nowadays, it is difficult to establish *a priori* whether a single or a combination of variants may impact on CI mechanism. In this frame, computational approaches can be used to support the experimental studies. Here, a computational approach based on coarse-grained molecular dynamics simulations was applied to investigate mutations on CI variants. Specifically, one of the primary CI variants involved in LHON onset (m.14484T>C/MT-ND6) [1] was analysed alone and in combination with two rare CI variants, whose role remains uncertain [2]. The primary variant, positioned in a fundamental region for CI function called E-channel, stiffens the enzyme dynamics. Moreover, one of the rare variants, located next to the primary one, seems to further worsens the stiffening, while the other probably does not affect CI function. This approach may be extended for future studies to other variants to analyse the pathogenic impact on CI dynamics, or to investigate multiple variants.

University of Bologna (Department of Pharmacy and Biotechnology) and CINECA consortium provided the resources for this project.

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Cite: Rigobello L, Lugli F, Caporali L, Bartocci A, Fadanni J, Zerbetto F, Iommarini L, Carelli V, Ghelli AM, Musiani F (2024) A computational study to investigate the pathogenicity of single or combinations of respiratory Complex I variants. Bioenerg Commun 2024.6:5. https://doi.org/10.26124/bec.2024-0006

Replenishing NAD+ content recovers morphofunctional aspects of mitochondria in the arcuate nucleus of obese mice

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Background: Excessive intake of saturated fats disrupts hypothalamic neuronal function, crucial for maintaining body energy balance. Mitochondrial activity within hypothalamic neurons is pivotal in regulating food intake and energy expenditure, dynamically adapting to cellular energy demands and nutrient availability. While neuronal activity is commonly studied in energy homeostasis, glial cells, particularly microglia, also sense brain environmental changes, providing structural and metabolic support and acting as the brain's immune system. In parallel, Nicotinamide Adenosine Dinucleotide (NAD+) plays a crucial role in mitochondrial function, yet its involvement in microglial-neuronal mitochondrial dynamics remains unclear. **Aim:** In this study, we examined the morpho functional aspects of mitochondria in the arcuate nucleus of the hypothalamus of obese mice treated with Nicotinamide Riboside (NR), a NAD+ booster. **Methods:** The cellular model was used to explore specifically microglial and neuron responses to palmitate treatment. We also examined the arcuate nucleus of the hypothalamus in HFD-fed and NR-treated mice, assessing physiological and molecular parameters through different techniques including RNAseq analysis, Western blot, mitochondrial respirometry and electron microscopy. **Results:** Our findings reveal that palmitate affected NAD synthesis and stimulates mitochondrial fragmentation in both, microglia (BV2 cells) and neurons (mHypho A2-29). Similarly, high fat diet consumption suppressed NAD synthesis pathway proteins, induced mitochondrial fragmentation and reduced mitochondrial respiration in the arcuate nucleus of mice. Conversely, the gene ontology biological processes analysis from RNAseq of arcuate nucleus revealed that NR treatment stimulated several mitochondrial-related pathways including NAD metabolism and oxidative phosphorylation. Finally, oral NR treatment effectively restored NAD markers, stimulated mitochondrial fusion and increased mitochondrial respiration capacity in the arcuate nucleus of mice. **Conclusion:** These preliminary data suggest that while saturated fatty acid affect NAD synthesis and mitochondrial metabolism in microglial cells neurons, NAD+ precursor may recover mitochondrial morpho functional aspects of these cells in the arcuate nucleus of obese mice.

Cite: Braga R, Katashima C, Rocha M, Pauli J, Silva A, Cintra D, Ropelle E (2024) Replenishing NAD+ content recovers morphofunctional aspects of mitochondria in the arcuate nucleus of obese mice. Bioenerg Commun 2024.6:6. https://doi.org/10.26124/bec.2024-0006

Structure of Vo domain of V/A-ATPase and MD simulation for rotation of rotor ring by proton motive force.

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The molecule adenosine triphosphate (ATP) serves as the central energy currency of life. The majority of ATP is synthesized by oxidative phosphorylation, catalyzed by ATP synthase and dependent on generation of the proton motive force across the membranes (*pmf*) by respiratory complexes in animal cells and bacterial cells.There are two types of ATP synthases: the F type (F_oF_1) and the V type ATP synthases. Similar to F-ATPase, the V/A-ATPase from *T. thermophilus* (*Tth*) synthesizes ATP utilizing *pmf* in contrast to eukaryotic V-ATPases which function as proton pumps powered by ATP hydrolysis.

In this study, we determined the structures of the V_o domain of the full V/A-ATPase and the isolated V_o domain using cryo-electron microscopy. The structure of the V_o domain of V/A-ATPase was almost identical to that of the isolated V_o. By analyzing the structure of the 2.8 Å resolution V_o, we indicated precise identification of glutamate residue (Glu) side chain orientations within the c_{12}-ring.MD simulations based on this structure revealed an asymmetric protonated state of the Glu residues in the two half-channels by the *pmf*. We propose a model in which the asymmetric protonation states of the Glu residues of the *c*-subunits on either side of the two channels provide the unidirectional bias for the Brownian motion of the c_{12}-ring.

Cite: Yokoyama K, Kishikawa J, Nishida Y, Nakano A, Okazaki K (2024) Structure of Vo domain of V/A-ATPase and MD simulation for rotation of rotor ring by proton motive force. Bioenerg Commun 2024.6:7. https://doi.org/10.26124/bec.2024-0006

Cell geometry and membrane protein crowding are major constraints on phenotype

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Bacterial cell geometry is highly regulated and constrains the surface area available for acquiring nutrients as well as the volume available for synthesizing proteins. The surface area to volume (SA:V) ratio of all documented bacteria decreases with growth rate due to increases in cell size. However, the membrane protein content of *Escherichia coli* increases with growth rate creating a positive correlation between membrane enzyme capacity and growth rate. Despite its central role in cell biology, the intersection of membrane protein capacity, cell geometry, and central metabolism has not been defined with a predictive and quantitative theory. Here, we present a biophysical basis for maximum growth rate, overflow metabolism, electron transport chain efficiency, and maintenance energy flux. The theory successfully predicts the phenotypes of two *E. coli* K-12 strains, MG1655 and NCM3722, which are genetically similar but have different SA:V ratios, different maximum growth rates, and different overflow phenotypes. These analyses do not consider the cytosolic proteome, demonstrating the predictive power of the surface area and membrane protein crowding alone. Analyses suggest *E. coli* does not maximize growth rate nor biomass yield. Instead, *E. coli* phenotypes operate at intermediate growth rates and yields while maximizing the areal density of ATP synthase complexes, maximizing the rate of substrate energy dissipation. Cell geometry and membrane protein crowding plays a central role in phenotype from prokaryotes to eukaryotic organelles.

Cite: Carlson R, Beck A, Gedeon T (2024) Cell geometry and membrane protein crowding are major constraints on phenotype. Bioenerg Commun 2024.6:8. https://doi.org/10.26124/bec.2024-0006

CryoEM evaluation of COX7A isoforms

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The physiological adaptability strategy of complex IV (cytochrome c oxidase) is handling several isoforms encoded by paralog genes that may vary according to different tissues, oxygen tension. Among these, subunit COX7A presents three different isoforms that can be alternatively selected for CIV assembly, and each one has a specific impact on the functionality of the OXPHOS system by affecting the ability of CIV of forming monomers, dimers or supercomplexes. However, the mechanism of selection of the different isoforms and how they modulate metabolism remains unclear.

On the other hand, one of these tissues where the organization of the electron transport chain (ETC) complexes presents high sensitivity to changes in isoforms is brown adipose tissue (BAT). In BAT, the supercomplexes formation is notably affected by the presence or absence of the different isoforms of COX7A. Furthermore, it has been described that cold exposure can affect supercomplex formation in BAT by rotating CIII in the interaction between CIII and CI. Therefore, to gain a better understanding of the underlying mechanisms for isoform selection and functionality we are performing computational analysis that combine genetic and structural information of the ETC complexes. In this project, we use high-precision cryo-electron microscopy (CryoEM) evidence to study isoform selection in CIV and its effects on the organization of the ETC complexes and supercomplexes formation.

Thus, we analyzed supercomplex I+III2+IV (also called N-respirasome) from BAT by Cryo-EM. We aim to obtain a structural model of the N-respirasome complex in which we explore the presence of each of the COX7A isoforms. At the same time, we are evaluating the effect of cold exposure that has been observed in CIV and CIII binding in our models of N-respirasome . Therefore, in our images, we expect to observe four populations of respirasomes. We contemplate to find respirasomes that present SCAF1, in which all the complexes are bound between them, and respirasomes where CIII and CIV are both bound to CI but not to each other. Also, we expect to see the effect of cold exposure affecting these respirasomes in different ways, as the movement of CIII respect to CI will affect CIV only when they are covalently bound by SCAF1.

Cite: Rosa-Moreno M, Cabrera-Alarcon JL, Enriquez Dominguez JA (2024) CryoEM evaluation of COX7A isoforms. Bioenerg Commun 2024.6:9. https://doi.org/10.26124/bec.2024-0006

Mitochondrial free radical detection via modulation of photoluminescence from nitrogen vacancies in diamond-based quantum sensors

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Mitochondria, essential for cellular viability, are the primary source of free radicals, crucial molecules with high reactivity and dynamic nature, making their detection challenging. This work presents a robust and straightforward quantum sensing method for selective free radical detection throughout biological processes. We employ negative charge state of Nitrogen-Vacancy (NV) colour centres in diamond exploiting their spin-dependent photoluminescence. We employ a low optical power method to harness the NV ground state spin triplet's sensitivity to paramagnetic species, like free radicals, that preserves the NV charge state. Using simple modifications to an inverted fluorescent microscope we perform Optically Detected Magnetic Resonance (ODMR) and Microwave Modulation (MM) of the NV photoluminescence and evaluate changes ODMR and MM contrast, as surrogate measures of the NV optical polarizability and hence paramagnetic species. We introduce a methodology using the spin probe 4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl (TEMPOL) for selective free radical identification. TEMPOL, a cell-permeable probe, scavenges free radicals, as a Superoxide dismutase 2 (SOD2) mimic, quenching the contrast in both ODMR and MM. Restoration of contrast is observed upon free radical species that preferentially react with TEMPOL (hydroxyl radical, superoxide radicals). Validation experiments with irradiation of H_2O_2 corroborated the findings.

The aforementioned methodology was employed in biological studies encompassing neuroblastoma cells, and wildtype versus PINK1 (PINK189/Y) whole *Drosophila Melanogaster*. PINK1 mutations exhibit a set of relevant phenotypes of Parkinson's Disease such as impaired locomotor activity, dopaminergic neuron degradation and mitochondrial abnormalities. High-resolution respirometry alongside specific substrate-uncoupler-inhibitor titrations with TEMPOL was performed to monitor mitochondrial function. This approach effectively detected free radicals across the electron transport system (ETS). Furthermore, the method distinguished enhanced free radical expression in PINK1 flies across the ETS compared to wildtype flies. This simple protocol offers significant advantages for cell biologists and medical discovery, lowering the barrier to entry for quantum sensing applications. This approach allows for studying the entire oxidative phosphorylation process and spin-active intermediates within biological systems. The proposed technology has the potential to help elucidate the biophysical parameters underlying mitochondrial function and dysfunction.

Cite: Reed J, Ebanks B, Menon S, Moisoi N, Mather M, Chakrabarti L (2024) Mitochondrial free radical detection via modulation of photoluminescence from nitrogen vacancies in diamond-based quantum sensors. Bioenerg Commun 2024.6:10. https://doi.org/10.26124/bec.2024-0006

Unravelling the molecular mechanisms of *Toxoplasma gondii* Complex II: Progress and challenges

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The four mitochondrial respiratory complexes (II, III, IV and V) of *Toxoplasma gondii* are essential for energy metabolism and survival. Of particular interest, is *T. gondii* complex II (succinate dehydrogenase, *Tg*CII) due to it being one of the entry points to the electron transport chain (with the absence of complex I from *T. gondii*) and the only link to the TCA cycle. Our previous studies have proposed seven putative new subunits in *Tg*CII for a total of nine, more than the usual four subunits found in mammals and yeast [1]. The contribution of these subunits to *Tg*CII have been experimentally validated through microscopy, cell-biology, and biochemical methods [2], raising the question of what the functional implications of the new complex composition are. Importantly, two of the canonical four subunits, that make critical contribution to the catalytic sites, don't seem to have clear homologs in the *T. gondii* complex. Furthermore, structural predictions do not produce a structure that includes the required active sites for *Tg*CII known activity. Sequence alignments provided some progress by revealing that one of the subunits, S10, contains a highly conserved DY motif involved in activity, and this was validated via genetics. To complete the picture and elucidate the molecular mechanisms of *Tg*CII function, we plan to solve the structure using single particle analysis via cryoEM. Here I show my progress in optimising purification of *Tg*CII, and of the data analysis pipelines via single particle analysis.

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Cite: Douglas K, Silva M, Maclean A, Meir A, Bhella D, Sheiner L (2024) Unravelling the molecular mechanisms of *Toxoplasma gondii* Complex II: Progress and challenges. Bioenerg Commun 2024.6:11. https://doi.org/10.26124/bec.2024-0006

Formation of I_2+III_2 supercomplex rescues respiratory chain defects

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According to the fluidity model, complexes of the mammalian mitochondrial electron transport chain (ETC) partition between free complexes and quaternary assemblies known as supercomplexes (SCs). However, the physiological requirement for SCs in oxidative metabolism is a matter of ongoing intense debate, and the mechanisms regulating their formation remain enigmatic. Here, we show that genetic perturbations affecting the biogenesis or maturation of mammalian ETC Complex III (CIII) stimulates the formation of a specialized extra-large SC (SC-XL) with a structure of I_2+III_2, resolved to a nominal resolution of 3.7 Å by cryogenic electron microscopy. SC-XL formation increases mitochondrial cristae density and sustains normal ETC output despite a 70% reduction in electron flow through CIII, effectively rescuing mild to moderate CIII deficiency. Increasing the SC-XL:free III_2 ratio significantly reduced CIII ROS production and propensity for CI ROS triggered by reverse electron transport, whereas inhibiting SC-XL formation via the Uqcrc1$^{DEL:E258\text{-}D260}$ mutation increased CIII ROS production and led to respiratory decompensation in CIII mutants. Furthermore, higher SC-XL:free III_2 ratio reprogrammed mitochondria towards fatty acid oxidation and protected against ischemic heart failure in mice. Our study reveals an unanticipated plasticity in the mammalian ETC to buttress against intrinsic perturbations via structural adaptations, and suggests that ETC reprogramming via controlled regulation of SC-XL formation is a potential therapeutic strategy for remediating diseases characterized by a decline in ETC bioenergetics and oxidative damage.

Cite: Liang C, Zhang S, Padvannil A, Beh S, Robinson D, Meisterknecht J, Cabrera-Orefice A, Koves T, Watanabe C, Watanabe M, Illescas M, Lim R, M. Johnson J, Ren S, Wu Y-J, Kappei D, Ghelli AM, Funai K, Osaka H, Muoio D, Ugalde C, Wittig I, Stroud D, A. Letts J, Ho L (2024) Formation of I_2+III_2 supercomplex rescues respiratory chain defects. Bioenerg Commun 2024.6:12. https://doi.org/10.26124/bec.2024-0006

Mitochondrial peptide SCARI regulates supercomplex assembly via Complex I turnover

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The electron transport chain complexes in the mitochondrial inner membrane (mtIM) associate to form supra-molecular assemblies known as supercomplexes (SCs). SCs have major biomedical implications as defects in respiratory complexes contribute to a broad spectrum of diseases. While the existence of SCs is now widely accepted, the molecular mechanisms regulating its assembly and stability remain elusive. We report the finding of SCARI, a small-ORF encoded peptide localized in the mtIM with functions in regulating the SC formation. In C2C12 myoblasts, SCARI forms a supra-molecular complex, and its deletion increases the steady state levels of Complex I and associated SCs. Deficiency of SCARI enhances mitochondrial respiration and confers growth advantage under oxidative stress conditions. We have defined the interactome of SCARI and identified Prohibitin-AFG3L2 (m-AAA protease) complex and Complex I subunits to be the major binding partners. Here, we propose that SCARI acts as an adaptor within prohibitin complex to regulate the turnover of Complex I subunits via AFG3L2 protease. Clarifying the regulatory mechanisms of SCARI in SC assemblies will aid in understanding the roles of OXPHOS defects in disease pathogenesis and to develop SCARI based therapeutics for mitochondrial diseases characterized by destabilized respiratory Complex assembly.

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Structural insights into the assembly of Complex IV

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The mitochondrial oxidative phosphorylation (OXPHOS) system consists of four enzymatic multiprotein complexes that collaborate to facilitate the transfer of electrons from reducing equivalents to molecular oxygen. Given the central role of OXPHOS in cellular energy metabolism, deficiencies in the enzymes catalyzing these processes contribute to human diseases. Understanding the assembly, regulation, and functioning of these complexes is crucial for unraveling the mechanisms underlying cellular energy metabolism and its implications for various health conditions.

In this study, the human respirasome CICIII2CIV was isolated from human cells, and high-resolution structures of the individual complexes were determined by cryo-EM. Intriguingly, we identified the presence of the HIGD2A protein, which binds to complex IV within the respirasome. Detailed structural analyses uncovered the mutually exclusive appearance of HIGD2A and NDUFA4 in complex IV. Our data propose a role for HIGD2A in the final stages of complex IV assembly, including the regulation of NDUFA4 incorporation, providing insights into the temporal dynamics of the assembly process.

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Deciphering pathological phenotypes associated with a single large deletion of mtDNA by *in vitro* modelling in cardiomyocytes and transcriptomic profiling

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Mitochondrial disorders are multisystemic diseases mainly associated with neurological features, but cardiac manifestations are also common traits and can be the major cause of significant comorbidity and mortality [1]. Kearns-Sayre syndrome (KSS), a heterogeneous neurodegenerative disorder in which many patients require pacemaker implantation, is caused by the clonal expansion of mutant mitochondrial DNA (mtDNA) with a single heteroplasmic large-scale deletion (common deletion). Here, we used patient-specific induced pluripotent stem cells (hiPSCs) derived from skin fibroblasts of an 8-year-old male patient carrying the common deletion, to generate cardiomyocytes (CMs). We obtained two clones with different levels of heteroplasmy, 60%, and 0% respectively, with the latter serving as an isogenic control to assess whether nuclear DNA can influence the pathological phenotype. As an additional control, CMs from an unrelated healthy subject were differentiated. When compared to isogenic and unrelated controls, the clone carrying the deletion had a lower oxygen consumption rate, a higher spontaneous beating rate with a shorter repolarization duration measured with MultiElectrode Arrays (MEAs) and a higher propensity for arrhythmias such as delayed afterdepolarizations (DADs) measured with patch clamp. Preliminary data from calcium transient measurements (Fluo-4AM) linked the deletion with a shorter calcium transient duration and with higher propensity for abnormal calcium transients. RNAseq analysis was performed to clarify which pathways might be involved in the pathogenic mechanisms. The main dysregulated pathways were: OXPHOS, calcium homeostasis, solute carrier transporters, vesicular trafficking and mitophagy. To investigate the presence of adaptive mechanisms, such as mitochondrial biogenesis, mtDNA copy number was measured by qPCR, which indicated the presence of increased level of mtDNA in deleted CMs as compared to isogenic and unrelated controls The ultrastructural morphology of mitochondria showed increased mitochondrial size.

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Suppressing hydrogen sulfide production affects mitochondrial biogenesis and mitochondria function in breast cancer

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Background: In humans, three enzymes; cystathionine-β-synthase(CBS), cystathionine gamma-lyase(CSE), and 3-mercaptopyruvate sulfurtransferase(3-MST) mainly synthesize the gasotransmitter hydrogen sulphide(H_2S). The expression of these enzymes was found to be significantly upregulated in breast cancer(BC). A key target of H_2S is the mitochondria, where H_2S has been demonstrated to affect mitochondrial respiration, morphology, function and biogenesis.H_2S was shown to upregulate or activate key players in the mitochondrial biogenesis pathway such as increasing PGC1α expression and activating NRF2. These effects were reported in hepatocytes and cardiac tissue. However, the role of H_2S on mitochondrial biogenesis in BC was not thoroughly examined. Thus, the aim of this study was to elucidate the role of mitochondrial biogenesis in BC patients and the impact of knocking down of H_2S synthesizing enzymes on mitochondria in BC cell lines.

Methodology: Breast tissues were collected from 26 female BC patients. TNBC MDA-MB-231 cells were transfected with CBS, and CSE siRNAs. RNA extraction was followed by reverse transcription into cDNA using reverse transcriptase.PGC1-α, Nrf1, NRF-2, TFAM and ND-1 expression was quantified using q-RT-PCR. Mitochondrial membrane potential was measured using rhodamine123 and ATP using luminescence kit.

Results: CBS, CSE, 3-MST and ND-1 were upregulated in BC tissues compared to the surrounding non-cancerous tissue. Knocking down of CBS in MDA-MB-231 cells resulted in an increased expression of PGC1α, NRF1/2, TFAM and ND-1. Similar results were observed with CSE knocking down except TFAM levels were not altered. Silencing of CBS and CSE reduced the mitochondrial membrane potential and decreased ATP levels in TNBC cells.

Conclusion: This study highlights the supportive role of H_2S in mitochondrial biogenesis and function in BC.

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Metformin restores mitochondrial bioenergetics, activates AMPK/PGC-1α pathway, and modulates mitochondrial dynamics in MOCS1-deficient fibroblasts

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Sulfite oxidase (SO) deficiency may arise from mutations in the *SUOX* gene, which encodes SO (isolated SO deficiency, ISOD), or mutations in the genes encoding enzymes involved in the biosynthesis of the molybdenum cofactor (molybdenum cofactor deficiency, MoCD). Mutations in *MOCS1*, *MOCS2* and *GPHN* result in MoCD type A, B and C, respectively. Both ISOD and MoCD are biochemically characterized by tissue accumulation of sulfite. Patients present with severe neurological symptoms and brain abnormalities, the pathophysiology of which is not fully established. Considering that mitochondrial dysfunction has been shown in different animal models for SO deficiency and that metformin induces mitochondrial biogenesis, we evaluated the effects of this molecule in fibroblasts from a patient with MoCD.

Fibroblasts derived from a patient with MOCS1 deficiency (MoCD type A) were incubated with metformin (2.5 or 5 μM) for 12, 24, and 48 hours in a medium devoid of glucose. After incubation, mitochondrial respiration was determined using the Seahorse Extracellular Flux Analyzer. Expression levels of genes and content of proteins involved in mitochondrial biogenesis and dynamics were determined by RT-qPCR and western blotting respectively.

A reduction in basal, maximal, and ATP-linked respiration and reserve respiratory capacity was verified in MOCS1-deficient fibroblasts. The protein content of MFN1/2, OPA1, and DRP1 were reduced in these cells. In addition, metformin treatment increased mitochondrial respiration. Furthermore, expression levels of *PRKAA1*, *PPARGC1A*, and *SIRT1* were upregulated after 24 hours of metformin incubation. Moreover, mRNA expression levels of *mitofusin 1* and *DNM1L* were enhanced in deficient cells following 48 hours of metformin treatment. Additionally, metformin increases MFN1/2, OPA1, and DRP1 protein levels in *MOCS1*-deficient fibroblasts.

Our data show mitochondrial respiration disruption along with mitochondrial biogenesis and dynamics disturbances in MOCS1-deficient fibroblasts. Moreover, metformin increased mitochondrial respiration and levels of mitochondrial biogenesis and dynamics proteins, suggesting that this molecule activates AMPK/PGC-1α pathway in these cells. Therefore, metformin is a potential adjuvant therapy for the treatment of patients with ISOD and MoCD.

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Knockout of the mitochondrial Complex III ubiquinol-cytochrome c reductase hinge protein inhibits acute but not chronic oxygen sensing in the pulmonary vasculature

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Background: Acute alveolar hypoxia triggers hypoxic pulmonary vasoconstriction (HPV) which is essential to optimize arterial oxygenation. In contrast, chronic hypoxia induces pulmonary vascular remodelling and consequently pulmonary hypertension (PH). HPV, but not PH, is triggered by increased superoxide release from mitochondrial complex III. As ubiquinol-cytochrome c reductase hinge protein (Uqcrh) deficiency reduces complex III activity, we hypothesized that *Uqcrh* knockout (*Uqcrh*$^{-/-}$) may impair acute oxygen sensing in the pulmonary vasculature.

Methods: HPV was quantified in isolated perfused and ventilated lungs from *Uqcrh*$^{-/-}$ and wild type (WT) mice. Acute hypoxia-induced cellular membrane depolarization was determined in pulmonary arterial smooth muscle cells (PASMC) from *Uqcrh*$^{-/-}$ and WT mice using patch clamp. To assess the role of Uqcrh in chronic hypoxic signalling, protein levels of the hypoxia-inducible factor 1α (HIF-1α) and cellular proliferation were determined in *Uqcrh*$^{-/-}$ and WT PASMC after exposure to normoxia or 1% oxygen (for 24h or 72h).

Results: HPV was inhibited in lungs from *Uqcrh*$^{-/-}$ mice, while pulmonary vasoconstriction induced by the thromboxane analogue U46619 was preserved. Acute hypoxia-induced cellular membrane depolarization was decreased in *Uqcrh*$^{-/-}$ PASMC compared to WT PASMC. In contrast, chronic hypoxic exposure increased HIF-1α protein expression and cellular proliferation to similar levels in *Uqcrh*$^{-/-}$ and WT PASMC. Furthermore, Uqcrh expression was not altered in WT PASMC after chronic hypoxic exposure.

Conclusion: These results support previous findings that acute and chronic hypoxic signalling is triggered by different mechanisms in PASMC. Electron flow through complex III is involved in acute, but not chronic oxygen sensing of the pulmonary vasculature.

Cite: Li M, Pak O, Alebrahimdehkordi N, Knoepp F, Giordano L, Hadzic S, Gailus-Durner V, Hrabě de Angelis M, Seeger W, Grimminger F, Schermuly RT, Weissmann N, Sommer N (2024) Knockout of the mitochondrial Complex III ubiquinol-cytochrome c reductase hinge protein inhibits acute but not chronic oxygen sensing in the pulmonary vasculature. Bioenerg Commun 2024.6:18. https://doi.org/10.26124/bec.2024-0006

Theory of torques and fast states in single-molecule observation of a rotary motor

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We present a method to analyze fast rotation trajectories in F1-ATPase using the distribution of angular velocity. The analysis involves the transitions during the stepping between pauses. A theoretical-computational approach is used to model the fluctuation of the imaging probe as the molecular motor undergoes stepping rotation. This method relies on the concept of an angle-dependent velocity and torque which are extracted from experimental data and calculated from theory. When applying the method on Thermophilic Bacillus F1-ATPase rotation data, we detected the presence of a short-lived substep previously not detectable in the histograms. The comparison between the experimental and theory reveals that an 80^0 substep of the "concerted" ATP binding and ADP release involves an intermediate state reminiscent of a 3-occupancy structure. Its lifetime (~10 μs) is about six orders of magnitude smaller than the lifetime for spontaneous ADP release. By detecting this short-lived state the method provides "temporal super-resolution". Most recently, this method was applied to single-molecule imaging data from Paracoccus Denitrificans F1-ATPase and it yielded a similar hidden state in the transitions between dwells. Our recent findings indicate a common mechanism for the acceleration of ADP release in the F1-ATPase motor of the two species.

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The 6 B's of CoQ_{10} therapeutic development: BPM31510, bioenergetics, biophysics, bench, bedside, and back again

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Ubidecarenone (CoQ_{10}) is a critical metabolic coenzyme that intricately reconnects dysfunctional metabolic homeostasis and offers biological connectivity for cellular governance of metabolic pathways, coordination of cell death, regulation of ROS, and cellular function. Due to the highly insoluble nature of CoQ_{10}, non-optimized formulations have resulted in poor absorption of CoQ_{10} into systemic circulation, leading to numerous failures in achieving a therapeutic benefit from CoQ_{10} administration.

BPM31510 (a novel lipid nanodispersion of ubidecarenone) is highly stable, possesses unique biophysical properties and able to deliver supraphysiological concentrations of oxidized CoQ_{10} under IV administration. Notably, the formulation demonstrates potent metabolic activity and potentiation of mitochondrial OXPHOS and ROS production, conferring actionable therapeutic potential across multiple disease phenotypes. The role of CoQ_{10} in redox homeostasis, free radical/ROS generation, and mitochondrial efficiency in a panapology disease states to include rare diseases such as epidermal bullosa and CoQ_{10} deficiency/other mitochondrial disorders, oncology (glioblastoma multiforme, pancreatic adenocarcinoma) as well as sarcopenia, are currently being investigated with BPM31510.

The clinical and translational insight gained from BPM31510 therapeutic development suggests that CoQ_{10} homeostasis in regulation of mitochondrial redox state, ROS, membrane biophysics, and bioenergetics as characterized by omics and spatial omics technologies has overcome the pK challenges in therapeutic development of CoQ_{10} delivery through the translational efforts in the development of BPM31510.

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Mitochondrial medicine in the pandemic era

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The common diseases affect the most energetic organs of the body: brain, heart, muscle, kidney, liver, and endocrine systems. The predominant source of energy is mitochondrial oxidative phosphorylation (OXPHOS), the genes of which are dispersed across the chromosomes and the multicopy, maternally inherited, mitochondrial DNA (mtDNA). The mtDNA codes for 13 critical OXPHOS genes, the remaining ~160 OXPHOS genes being encoded in the nuclear DNA (nDNA). Expression of mtDNA and nDNA genes is coordinated by alterations in mitochondrial metabolic intermediates which are the substrates for the epigenomic histone and nDNA modification enzymes. Inhibition of OXPHOS causes increased mitochondrial reactive oxygen specific (mROS) production, and elevated mROS can damage the mtDNA and mtRNA which are released into the cytosol to activate the innate immune system: inflammasome, cGAS-STING interferon pathway, and toll-like receptor 9. Therefore, OXPHOS inhibition results in an array of complex diseases ranging from diabetes to cardiovascular disease to neurological diseases including autism and Alzheimer disease, all associated with inflammation.

Environmental factors can impinge on mitochondrial function. In COVID-19, SARS-CoV-2 infection suppresses the transcription of clusters of nDNA genes required for specific subassembly modules of the OXPHOS complexes, thus blocking OXPHOS. SARS-CoV-2 also induces miR2392 which binds to the mtDNA blocking its transcription. Viral OXPHOS inhibition increases mROS which activates HIF1α to shift metabolism from OXPHOS to glycolysis, thus redirecting substrates to viral biogenesis. Specific SARS-CoV-2 coded polypeptides function to alter host nDNA OXPHOS gene expression which can be sustained long after the virus has been cleared. Continued suppression of nDNA OXPHOS gene expression has been documented in heart, kidney, and liver of COVID-19 autopsy specimens and may contribute to long-COVID [1].

Given the central role of mROS and HIF-1α activation in SARS-CoV-2 metabolic remodeling [2], we reasoned that treatment of SARS-CoV-2 infected mice with mitochondrially-targeted catalytic antioxidants should inhibit SARS-CoV-2 propagation and pathogenicity. Accordingly, systemic expression of mitochondrially-targeted catalase (mCAT) or treatment with EUK8 markedly impaired SARS-CoV-2 gene expression, lung inflammation, and systemic mouse pathology. Since treatment with mitochondrial antioxidants alters the host not the virus, this therapeutic approach should be resistant to SARS-CoV-2 S protein mutations that subvert current immunization [3].

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Photoprotective strategies in *Chlamydomonas reinhardtii*: The role of flavodiiron proteins under contrasting oxygen tensions

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Almost all life on earth depends on photosynthesis, which is the basis of agricultural food and feed production. Regulation of photosynthesis is fundamental for efficiency and coping with changing environmental factors, preventing excess light energy from causing oxidative damage. Alternative electron pathways are crucial "safety valves" under stressful conditions, including changes in irradiation, nutrient availability and potentially high oxygen tensions. Algae use flavodiiron proteins (FDP) to protect photosystem I (PSI) by diverting excess electrons to reduce oxygen to water without forming reactive oxygen species (ROS). In *Chlamydomonas reinhardtii*, they are considered to protect PSI during a dark to light transition when the Calvin-Benson cycle is not active, or under fluctuating light. Oxygen tensions of the water column can vary considerably; especially dense algal populations exposed to high light and CO_2 concentrations encounter hyperoxia due to high photosynthetic rates. Here, using high precision respirometry, PSI absorption measurements (P700) and an *FDP* knockout (*flvb*), we further characterised FDP activity and its protective role. Near-infra red absorbance measurement of the PSI reaction centre (P700) of *C. reinhardtii*, indicated that hyperoxia led to P700 oxidation during a saturating pulse in wild-type, but not in an FDP-deficient mutant (*flvb*). We therefore hypothesized that FDP play a role under hyperoxia and saturating light intensities, protecting from excess Mehler reaction and ROS formation. While results supported our hypothesis, to our surprise we found that under constant sub-saturating light intensity that FDP is actually important in the protection of PSII [1].

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MIMAS, a mitochondrial multifunctional mega-assembly

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The powerhouse of the cells, mitochondria, are highly dynamic double-membrane organelles. The protein-rich nature of mitochondrial inner membrane and its important functions in respiration and mitochondrial biogenesis require a high degree of organization and flexibility. The inner membrane is home to several well-established protein complexes that are subject to compartmentalization, including the respiratory chain (super)complexes, the prohibitin complex, and TIM translocases. These protein complexes play fundamental roles in mitochondrial biogenesis, homeostasis, regulation, and physiology. Here we present a recently identified inner membrane mega-assembly, mitochondrial multifunctional assembly (MIMAS) [1]. Striking features of the MIMAS complex include its large size of around 3 MDa, as well as its dependence on the phospholipid phosphatidylethanolamine. By combining multiple methods, like biochemical approaches, analysis of the high-resolution mitochondrial complexome [2], and mass spectrometry, we were able to identify MIMAS components involved in diverse functions such as respiratory chain assembly, lipid biosynthesis and metabolic processes. Our results suggest that this protein-lipid assembly functions as a biogenesis platform that may organize the inner membrane by integrating different aspects of mitochondrial physiology.

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Mitochondria bound to lipid droplets: Where mitochondrial dynamics regulate lipid storage and utilization

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Are all mitochondria equal? No, they aren't. Here is an example: Mitochondria surrounding lipid droplets (Peri-Droplet Mitochondria; PDM) maintain a unique proteome that is not equilibrated with the rest of the mitochondrial population of the same cell. We find that PDM remain on the lipid droplet and do not fuse with cytosolic mitochondria. Compared to cytosolic mitochondria, PDM have a superior capacity to metabolize pyruvate and a reduced capacity to oxidize fatty acids. PDM are found in cells that build up lipid droplets. ATP synthesized by PDM fuels lipid droplet expansion.

In contrast, cytosolic mitochondria (CM) have a fragmented architecture and a superior capacity to utilize fat as a fuel source. The association of fragmented architecture with increased fat utilization intrigued us for a while, as we have observed the same in beta cells, hepatocytes and cancer. In a study by Jenny Ngo in our lab, in collaboration with Danial, Divakaruni, and Liesa labs, we reveal the mechanism by which the elongated mitochondrial shape supports fatty acid utilization by mitochondria. We find that mitochondrial elongation reduces fatty acid utilization by inhibiting CPT1 activity. Conversely, we find that mitochondrial fragmentation, such as observed in NASH, increases mitochondrial lipid utilization and may act as a compensatory mechanism to reduce lipotoxicity. Indeed, in collaboration with Pocai's lab at Janssen, we find that inhibition of fission in a model of NASH, exacerbates the NASH phenotype. This study is a good example demonstrating that mitochondrial fission and fragmentation are required for healthy function and in some cases represent compensatory responses required to mitigate disease.

What makes mitochondria depart from or bind to lipid droplets came next. Rebeca Acin Perez, Doyeon Kim and Essam Assali in our group, and in collaboration with Sekler lab reconstituted mitochondria and lipid droplet binding in a cell-free system where we tested candidate attachment and detachment signals. Brown adipose tissue (a mitochondrial researcher's best friend) gave us a couple of hints. PDM can be induced to depart from lipid droplets rapidly and robustly by adrenergic stimulation. In fact, the cytosol isolated from brown adipose tissue can induce or prevent the attachment of mitochondria to lipid droplets. So, what is in that cytosol that does that? This cytosolic factor will be the highlight of my presentation.

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Bilirubin-induced modulation of mitochondrial respiration in HEK293T cells

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Mildly elevated concentrations of total bilirubin (BR), the end product of heme catabolism, are considered protective (e.g. up to 60 µM in individuals with Gilbert syndrome). However, high BR concentrations are toxic and pose risks, as evidenced by the adverse effects of newborn jaundice. BR can reduce mitochondrial respiration, initiate apoptosis, or induce changes in cell membranes [1]. Despite this, the specific effects of BR on mitochondrial respiration or respiratory complexes remain poorly understood.

This study aimed to elucidate the direct effects of toxic BR concentrations on mitochondrial respiration in Human Embryonic Kidney cells.

Mitochondrial respiration was measured using Oroboros O2k Oxygraphs (Oroboros Instruments, Innsbruck, Austria) with cryopreserved HEK293T cells and MiR05 respiration medium containing bovine serum albumin (1 g·mL^{-1}). The direct effect of BR was assessed during succinate-stimulated respiration in both the LEAK state (low adenylates, high mitochondrial membrane potential, MMP) and the OXPHOS state (high ADP, low MMP). After introducing HEK293T cells into the chamber (0.5–0.75·10^6 x·mL^{-1}), cells were permeabilized using digitonin (5 µg·mL^{-1}). Succinate (10 mM), rotenone (0.5 µM), and cytochrome *c* (10 µM) were titrated to initiate LEAK respiration, followed by the addition of ADP + Mg^{2+} (1 mM) to induce the OXPHOS state. BR (up to 35 µM dissolved in DMSO) was subsequently titrated to observe its direct effects on mitochondrial respiration in both the LEAK and OXPHOS states.

Our results indicated that BR at 35 µM had no effect on the mitochondrial respiration of HEK293T cells in the OXPHOS state. However, BR at concentrations from 15 µM significantly increased LEAK respiration in a concentration-dependent manner up to 30 µM suggesting dyscoupling the mitochondrial inner membrane (mtIM). The addition of cytochrome *c* significantly improved LEAK respiration at toxic bilirubin levels, suggesting defects also in the mitochondrial outer membrane (mtOM). However, this effect on mtOM was not observed in the OXPHOS state.

In conclusion, our data suggest that BR may affect mitochondrial membranes in the LEAK state. Further experiments are needed to clarify the mechanisms and to elucidate why BR did not have this effect in the OXPHOS state.

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MMP-7 mediated proteolytic processing mitochondria-targeting EGFR $^{T790M/L858R}$ C-terminal fragments involved in EGFR axes modulate cancer stem cell transformation

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EGFR pathway involved in cancer cell migration, proliferation and survival drives a lots attentions of cancer biologist in searching the therapeutic targets. Tyrosine kinase inhibitor (TKI)-resistant small lung carcinoma cells and recurrent cancer stem cell sub population with EGFR mutations have been quite frustrated approaches by anti-EGFR based therapy. In the synthetic EGFR mutant axils enlightening Mitochondria-desRed small lung carcinoma CL1-0 cell line revealing an interesting findings well correlating the active EGFR or spontaneous active EGFR $^{T790M/L858R}$ mutant with high energy demanding status. In our EGFR axes-mitochondria synthetic cell based model, we first observe a phenomenon that EGF treatment enhances the amount of mitochondria per cell which is correlated to up-regulation of MMP-7 expression. The MMP-7 mediated proteolytic processing substrates could potentially activate mitochondria proliferation. EGFR can be cleaved by MMP-7 and release proteolytic EGFR c-terminal fragments further translocated to mitochondria and initiate mitochondria proliferation. Over expression of C-terminal fragments of EGFR $^{T790M/L858R}$ mutant in CL1-0 can translocate into mitochondria, because through MMP-7 cleaving EGFR reveal the cryptic mitochondria targeting sequences which can drive C-terminal EGFR $^{T790M/L858R}$ trafficking into mitochondria and lead the bonifying proliferation of mitochondria. Over expression of C-terminal fragments of EGFR $^{T790M/L858R}$ mutant in CL1-0 can increase the capacity of soft-agar growth and also increasing the sphere growth in the floating colonies. All of these evidences indicate that both MMP-7 mediated proteolytic processing EGFR $^{T790M/L858R}$ releasing C-terminal fragments or over expression of C-terminal EGFR $^{T790M/L858R}$ translocating into mitochondria confirm the anchorage-independent growth in soft agar and sphere suspension growth capacity and implicates the cancer stem cell transformation. The platelet purified mitochondria transplantation into the C-terminal EGFR $^{T790M/L858R}$ mutant CL1-0 can induce cancer/cancer stem cell apoptosis. Mitochondria targeting compound 007 can also sensitize EGFR $^{T790M/L858R}$ mutant CL1-0 to gefitinib chemotherapy.

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Different catalytic states of plant cytochrome *b*$_6$*f* revealed by high-resolution cryo-EM structures

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Cytochrome *b*$_6$*f*, a multi-subunit enzyme, plays a crucial role in the photosynthetic electron transport chain, linking Photosystems I and II and facilitating the transfer of electrons between plastoquinone (PQ) and plastocyanin. This process is vital for the conversion of light energy into chemical energy during photosynthesis. Although the atomic structure of cytochrome *b*$_6$*f* has been elucidated, the intricate details of its catalytic mechanism have remained elusive and a subject of intense scientific investigation. In this study, we present new high-resolution cryo-EM structures of spinach cytochrome *b*$_6$*f*, which provide unprecedented insights into the molecular interactions and orientation of the substrate PQ within the enzyme's active quinone reduction site. Notably, our findings indicate that PQ binds to the active site in a manner distinct from that of known inhibitors. Instead, PQ adopts a unique orientation, suggesting a different interaction mode. We also captured cytochrome *b*$_6$*f* in different conformations, including various positions of the iron-sulfur protein (ISP). By elucidating these structural nuances, our research offers a deeper understanding of the dynamic mechanisms involved in quinone catalysis within cytochrome *b*$_6$*f*. This work not only enhances our comprehension of the fundamental processes underlying photosynthesis but also sheds light on the broader molecular mechanisms that sustain life on our planet, highlighting the intricate interplay between structure and function in biological systems.

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Non-canonical role of ubiquinone in maintaining morphology and function of mitochondrial DNA nucleoids

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Ubiquinone is an essential metabolite that greatly aids mitochondrial respiratory function. It is responsible for carrying electrons from respiratory complexes I and II to respiratory complex III, which leads to ATP production by oxidative phosphorylation. Ubiquinone is also known to act as an anti-oxidant by preventing damages from various reactive oxygen species. With its hydrophilic benzoquinone head and hydrophobic polyprenyl tail, ubiquinone mainly exists within the inner mitochondrial membrane.

The protein subunits of respiratory complexes are encoded by both mitochondrial DNA (mtDNA) and nuclear DNA. Thus the proper function and maintenance of mtDNA is indispensable for governing both mitochondrial and cellular activity. In live imaging of cultured mammalian cells, these mtDNA molecules are observed as dynamic complexes known as mitochondrial nucleoids. We previously showed that the dynamic nature of nucleoids, regulated in cooperation with mitochondrial membrane fusion and fission, is essential for maintaining mitochondrial respiratory complex formation. However, molecular details and the pathophysiological roles of the mitochondrial nucleoid dynamics remained ambiguous.

In this work, we revealed that ubiquinone has a unique function in regulating the functional expression of mtDNA in mitochondrial inner membrane. Loss of ubiquinone synthesis not only causes mitochondrial respiratory failure, as expected, but also results in the incomplete formation of respiratory complexes and deformation of mitochondrial nucleoid structures, which is unrelated to its traditional electron carrying and anti-oxidising roles. As a result, we found a non-canonical role of ubiquinone and propose the exploitation of this phenomenon towards therapeutic advantage in clinical conditions arising from mitochondrial defects due to insufficient mtDNA activity.

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Coenzyme Q (ubiquinone) at the crossroads of metabolic pathways in the mitochondrial respiratory system

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Coenzyme Q (CoQ, ubiquinone) plays a pivotal role in the mitochondrial respiratory system, acting as an electron carrier within the electron transport chain (ETC). CoQ serves as a substrate for various mitochondrial enzymes, facilitating electron transfer from NADH and succinate via Complexes I and II, respectively, to Complex III (cytochrome bc_1 Complex) [1]. This electron transfer is crucial for the production of ATP through oxidative phosphorylation. CoQ is utilized by several dehydrogenases, including NADH dehydrogenase (Complex I), succinate dehydrogenase (Complex II), dihydroorotate dehydrogenase, choline dehydrogenase, and mitochondrial glycerol-3-phosphate dehydrogenase, among others [1]. These enzymes are involved in diverse metabolic pathways such as amino acid and fatty acid oxidation, nucleotide biosynthesis, and hydrogen sulfide detoxification [1]. The interaction of CoQ with these enzymes is not merely diffusion-controlled but involves specific binding and electron transfer mechanisms. For instance, the reduction of CoQ by NADH:ubiquinone oxidoreductase (Complex I) follows a ping-pong kinetic mechanism, indicating a complex interaction between the enzyme and CoQ [2]. Additionally, the $CoQH_2$/CoQ ratio serves as a sensor for the efficiency of the respiratory chain, modulating the configuration of the ETC to match the metabolic demands and substrate availability [3]. This dynamic regulation ensures optimal electron flux and minimizes the generation of reactive oxygen species, thereby maintaining mitochondrial function and cellular health [3].

In summary, CoQ is integral to the mitochondrial respiratory system, interfacing with multiple enzymes to drive essential metabolic processes and regulate the efficiency of the electron transport chain [1-4].

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Snapshots of acetyl-CoA synthesis, the final step of CO_2 fixation in the Wood-Ljungdahl pathway

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In the ancient microbial Wood-Ljungdahl pathway, CO_2 is fixed in a multi-step process ending with acetyl-CoA synthesis at the bifunctional carbon monoxide dehydrogenase/acetyl-CoA synthase complex (CODH/ACS). Here, we present catalytic snapshots of the CODH/ACS from the gas-converting acetogen *Clostridium autoethanogenum*, characterizing the molecular choreography of the overall reaction including electron transfer to the CODH for CO_2 reduction, methyl transfer from the corrinoid iron-sulfur protein (CoFeSP) partner to the ACS active site and the acetyl-CoA production. Unlike CODH, the multidomain ACS undergoes large conformational changes to form an internal connection to the CODH active site, accommodate the CoFeSP for methyl transfer and protect the reaction intermediates. Altogether, the structures allow us to draw a detailed reaction mechanism of this enzyme crucial for CO_2 fixation in anaerobic organisms.

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Coenzyme Q redox poise in sarcopenic obesity

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Sarcopenic obesity, or the loss of skeletal muscle function mediated by age-related adiposity, is an increasingly prevalent disease with ineffective medical treatments and poor survival. Notably, the mechanisms underlying the onset and progression of sarcopenic obesity remain largely unclear. We established a mouse model of sarcopenic obesity by placing aged mice on a high fat diet (60% kcal) for 4 weeks which increased body fat while decreasing muscle function and lean mass relative to age-matched low fat diet controls. We employed 10 weeks of exercise training (3-4 day/week; 1 hr/day; 70% of max speed) as a benchmark to differentiate putative cellular mediators associated with gain and loss of muscle function in the context of sarcopenic obesity. Mice with sarcopenic obesity exhibited defective NADH- and succinate-linked oxidative phosphorylation (OXPHOS) capacity, which were entirely normalized by exercise training. Defects in oxidative capacity were restored experimentally by bypassing the Q pool with durohydroquinone, a coenzyme Q (CoQ) analogue and electron donor for Complex III. To test whether these observations occurred due to CoQ deficiency, oxidation of the Q pool, or inadequate electron flow to Complex III, mice with sarcopenic obesity were chronically administered mitoquinone mesylate (MitoQ), a mitochondrially targeted CoQ10 derivative that selectively reduces the Q pool without donating electrons to Complex III. 8 weeks of MitoQ treatment enhanced muscle function in mice with sarcopenic obesity to the level of an aged low fat diet control while restoring CoQ redox poise and NADH- and succinate-linked OXPHOS capacity. Importantly, mice with sarcopenic obesity displayed a deficiency in the absolute CoQ abundance, which was not rescued by MitoQ treatment. Taken together, the skeletal muscle CoQ pool becomes overly oxidized in mice with sarcopenic obesity, limiting OXPHOS capacity and muscular fitness. Restoring CoQ redox poise by exercise training or mitochondrially targeted CoQ therapy reverses the defects in OXPHOS capacity and locomotor function independent of CoQ abundance. Thus, maintaining CoQ redox poise represents a promising therapeutic strategy for sarcopenic obesity.

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Bioenergetic cost on zebrafish intestine caused by a ubiquitous pollutant, glyphosate

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Glyphosate is a widely known herbicide that has become a pervasive contaminant in aquatic ecosystems due to its extensive use in agriculture. Despite its widespread presence, glyphosate is often considered safe based on earlier toxicological assessments [1]. However, recent studies have increasingly highlighted its potential hazardous effects on aquatic organisms, which has raised significant concerns about its ecological footprint [2, 3]. To understand more of the physiological imvpact on aquatic organisms, we assessed the bioenergetic cost in the intestine of zebrafish (*Danio rerio*), following exposure to environmentally relevant concentrations of glyphosate.

We designed an experiment involving three groups: a control group and two treatment groups (exposed to low and high concentrations of glyphosate added to the rearing water). Each group had 5 replicates, with 15 adult fish per tank. After 21 days of exposure, intestinal tissues were collected to assess oxidative stress using standard plate-reader assays, mitochondrial membrane potential through fluorescence-based techniques, and mitochondrial respiratory functions via Oroboros™ O2k instrument. Preliminary results did not reveal any statistically significant differences for most parameters, except for a significant reduction in respiratory capacity of Complex I (CI), and Complex II (CII) in the glyphosate-exposed groups, pointing to potential mitochondrial dysfunction. These findings warrant further investigations to better understand the specific impacts on mitochondrial functions. Our ongoing research aims to further explore the mechanisms underlying mitochondrial dysfunction in zebrafish intestine when exposed to glyphosate.

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Explorative analysis of mitochondrial function in Long-COVID patients compared to a control group

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Long-COVID is a clinical condition characterized by long-term consequences of severe respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, which poses a persistent public health concern worldwide. It impacts multiple body functions, including immunological, respiratory, cardiovascular, gastrointestinal, neuropsychological, musculoskeletal, and other important systems. Affected individuals frequently describe symptoms such as “mental fog” and persistent fatigue, among other various symptoms. Despite intensified research, the biological mechanisms causing this multitude of symptoms are not fully understood, sparking a quest for innovative research directions, particularly in the combined area of bioenergetics and psychoneuroimmunology. This interdisciplinary field focusses on how (immune) cells generate energy, exploring possible bioenergetic disruptions in individuals with Long-COVID, especially focusing on the role of mitochondrial functioning in peripheral blood mononuclear cells (PBMC) isolated from whole blood.

This research adopts a comprehensive approach, starting with clinical evaluations to measure both mental and physical impairments in those impacted. Using a thorough methodology, it merges clinical assessments of psychological issues and the influence of body weight, with the gathering of blood samples for isolating PBMC. These cells are being analyzed applying O2K high-resolution respirometry to evaluate mitochondrial function, providing insights into cellular energy dynamics and potential malfunctions in Long-COVID.

Our findings indicates significant disruptions in the mitochondrial activity in intact PBMC collected from patients with Long-COVID compared to non-affected controls, indicating a major loss of cellular energy processes, at least in immune cells. Being under current investigation, these issues might also correlate with the clinical symptom severity of Long-COVID, such as severe fatigue and cognitive impairments. The final findings will be shared during the presentation.

We will highlight the essential importance of mitochondrial health in understanding and managing Long-COVID more broadly. Evidence for mitochondrial perturbations in PBMC points towards bioenergetic health as a key area for both tracking and addressing the wide range of Long-COVID symptoms. Expanding our grasp of the disease's biological basis not only deepens our understanding of Long-COVID, but also unveils new paths for developing specific interventions aimed at reducing the long-lasting impact of the ailment and asks for a holistic approach.

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Bioenergetic myths of cellular energy transduction

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The study of energy transduction in eukaryotic cells has been divided between Bioenergetics and Physiology, reflecting and contributing to a variety of Bioenergetic myths considered here: (*1*) ATP production = energy production, (*2*) energy transduction is confined to mitochondria (plus glycolysis and chloroplasts), (*3*) mitochondria only produce heat when required, (*4*) glycolytic ATP production is inefficient compared to mitochondrial, and (*5*) mitochondria are the main source of reactive oxygen species (ROS) in cells. These myths constitute a 'mitocentric' view of the cell that is wrong or unbalanced. In reality, mitochondria are the main site of energy dissipation and heat production in cells, and this is an essential function of mitochondria in mammals. Energy transduction and ROS production occur throughout the cell, particularly the cytosol and plasma membrane, and all cell membranes act as two-dimensional energy conduits. Glycolysis is efficient, and produces less heat per ATP than mitochondria, which might explain its increased use in cancer cells and exercising muscle.

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