to the adult samples. Ultrastructure studies by electron microscope imaging indicated a reduction in the density and packing of cristae in the senescent mice, compared to the adult population. We also found a decrease in the cristae width in senescent mice.

Together, our data suggests changes in the mitochondrial ultrastructure in the heart that may be related to bioenergetic adaptation in response to aging.

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S12.P46 Bi-Genomic Mitochondrial-Split-GFP – the yeast system for screening the mitochondrial matrix echoforms of dually localized proteins

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A single nuclear gene can be translated into a protein that distributes in many cellular compartments. Accumulating evidences show that a lot of yeast *Saccharomyces cerevisiae* mitoproteins have dual mitochondrial and another compartment localization. The differentially localized pools of such proteins have been named echoforms. Unraveling the existence of mitochondrial echoforms using current GFP (Green Fluorescent Protein) fusion microscopy approaches is extremely difficult, especially for the cytosolic proteins, because the GFP signal of the cytosolic echoform almost inevitably masks that of the mitochondrial one. We therefore engineered a yeast strain expressing a new type of Split-GFP system termed Bi-Genomic Mitochondrial-Split-GFP (BiG Mito-Split-GFP). Split-GFP is based on the partition of 11 beta strand-composed GFP into two fragments: one long fragment that encompasses the 10 first beta strands ($GFP_{\beta 1\text{-}10}$) and one smaller fragment that consists of the remaining beta strand ($GFP_{\beta 11}$). In this strain the $GFP_{\beta 1\text{-}10}$ fragment is expressed from the mitochondrial genome under the control of the *ATP6* promoter and translated inside the organelle without interfering with mitochondrial function. The $GFP_{\beta 11}$ is expressed from a plasmid under the control of a strong GDP promoter and can be fused to any nuclear-encoded protein that will be translated by cytosolic translation machinery. Both Split-GFP fragments are translated in separate compartments and only mitochondrial proteins or echoforms of dual localized proteins trigger GFP reconstitution and can be visualized by fluorescence microscopy of living cells. We could authenticate the mitochondrial importability of any protein or echoform from yeast, but also from other organisms such as the human Argonaute 2 mitochondrial echoform.

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S12.P47 Comparable respiratory activity in attached and suspended fibroblast cell lines

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Measurement of mitochondrial respiration of cultured cells is widely used for mitochondrial diseases diagnosis and research. Fibroblasts are easily obtained material from patients and therefore often used for experiments. They are cultured in monolayers, but physiological measurements are carried out in suspended or attached cells, which, therefore, presents not only a methodological challenge but provides insight into fundamentals of the cell biology. The aim of this study was to investigate whether respiration differs in attached versus suspended cells using multiwell respirometry (Agilent Seahorse XF24) and high-resolution respirometry (Oroboros O2k). Platform comparison of two respirometers was performed and subsequently mitochondrial respiration in attached and suspended fibroblasts was compared. Mitochondrial respiration measured in culture medium was baseline-corrected for residual oxygen consumption and expressed as oxygen flow per cell. No differences were observed between attached and suspended cells in ROUTINE respiration of living cells and LEAK respiration. The electron transfer capacity was higher in the O2k than in the XF24, possibly explained by a limitation to two uncoupler titrations in the XF24[1].Our data suggest that short-term suspension of fibroblasts did not affect respiratory activity and coupling control. This project gives an overview of the advantages of the two methods from various points of view and provides a practical comparison between two widely used respiratory methods.

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S12.P48 How does the presence of mitochondrial DNA regulate the cell cycle in *Saccharomyces cerevisiae*?

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Mitochondria play a key role in several cellular functions and their impairment may impact the whole cell metabolism and manifest in disease. The presence of mitochondrial DNA (mtDNA) is not essential for yeast cell survival, although its absence greatly impacts cellular metabolism. One of the adaptative responses to loss of mitochondrial DNA is a delay in cell cycle progression from G1 to S phase [1]. However, the initial signal that triggers the G1-to-S phase delay, as well as the pathway that mediates the signaling from the

mtDNA-deficient mitochondria to the cell-cycle-regulating machinery remain unknown.

Here, we show that decreased transmembrane potential across the inner mitochondrial membrane acts as the signal to slow down the G1-to-S transition in cells lacking, as well as containing, mtDNA. Accordingly, increased transmembrane potential in cells lacking mtDNA is sufficient to restore the timely progress from G1 to S phase. Moreover, we present a pooled fitness screening experiment to identify proteins involved in the signaling pathway mediating G1-to-S cell cycle delay in cells lacking mtDNA. The identification of the regulators of cell cycle progression in mtDNA-deficient cells may have implications for disease states involving mitochondrial dysfunction.

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S12.P49 Impaired development-associated metabolic switch in the brain of rat model of autism spectrum disorder

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Upon neuronal differentiation, the developing brain undergoes a metabolic switch to mitochondria-mediated respiration to support newly acquired specialized functions. We and others, have implicated mitochondria dysfunction in the pathophysiology of autism spectrum disorder (ASD). However, there is limited knowledge about disturbances in brain bioenergetics and brain substrate utilization in ASD at different developmental stages. In the current study, we utilized high resolution respirometry to explore mitochondrial respiratory activities during prenatal (Embryonic day 20 (E20)) and neonatal stages (postnatal day 0 (P0)), and postnatal day 15 (P15) and in adolescence (postnatal day 30 (P30)) in prefrontal cortex of rodent model of ASD induced by exposure to valproic acid in utero. In developing control brains both complex I and complex II-driven oxidative phosphorylation (OXPhos) as well as respiratory capacity were found to dramatically increase at P30 relative to E20, P0 and P15. However, the same parameters in ASD brains didn't differ to any significant extent which implies an impaired metabolic remodeling during the development of autistic brains. Interestingly, we found a strong trend of decreased complex I and complex II-dependent OXPhos in ASD brain relative to control brains at P15 and P30. Moreover, a significant decrease in respiratory capacity mediated by complex-I+II-dependent respiration in ASD brains relative to control brains at P15 and a strong trend of lower respiratory capacity in ASD brains when compared with control brains at P30, hinting at impaired mitochondrial respiratory function in ASD brains during the peak of brain maturation. We then investigated changes in brain lactate levels during development in control and ASD animals. We found age-dependent increase in brain lactate levels in control brain with significant increase in P30 when compared to E20 and P0. No significant differences have been detected in brain lactate levels in ASD animals at matching developmental phases. Interestingly, brain lactate levels in ASD animals were significantly lower relative to control animals at P15 and P30 hinting at enhanced utilization of lactate in ASD brains that persist at P15 and P30. Taken together, our findings demonstrate impaired metabolic switch to aerobic respiration in ASD brain during the peak of neuronal differentiation, driven by deficits in mitochondria-mediated oxidative phosphorylation and suggest that mitochondria dysfunction may contribute to the ASD phenotype, opening the possibility of mitochondria targeted therapies.

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