thioredoxin (Trx) donates electrons to peroxiredoxin (Prx) to remove H2O2 and then thioredoxin reductase (TrxR) maintains the reduced Trx concentration with NADPH as the cofactor. Despite a great deal of kinetic information gathered on the removal of H2O2 by the Trx system from various sources/species, a mechanistic understanding of the associated enzymes is still not available. We address this issue by developing a thermodynamically-consistent mathematical model of the Trx system which entails mechanistic details and provides quantitative insights into the kinetics of the TrxR and Prx enzymes. Consistent with experimental studies, the model analyses of the available data show that both enzymes operate by a ping-pong mechanism. The proposed mechanism for TrxR, which incorporates substrate inhibition by NADPH and intermediate protonation states, well describes the available data and accurately predicts the bell-shaped behavior of the effect of pH on the TrxR activity. Most importantly, the model also predicts the inhibitory effects of the reaction products (NADP+ and Trx(SH)2) on the TrxR activity for which suitable experimental data are not available. The model analyses of the available data on the kinetics of Prx from mammalian sources reveal that Prx operates at very low H2O2 concentrations compared to their human parasite counterparts. Furthermore, the model is able to predict the dynamic overoxidation of Prx at high H2O2 concentrations, consistent with the available data. The integrated Prx-TrxR model simulations show that the coupling of TrxR- and Prx-dependent reduction of H2O2 allowed ultrasensitive changes in the Trx concentration in response to changes in the TrxR concentration at high Prx concentrations.

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**Higher Mitochondrial Membrane Potential Induces ROS Production in the Familiar Form of Frontotemporal Dementia with *MAPT* Mutations**

**Noemi Esteras Gallego**, Selina Wray, Elisavet Preza, Andrey Y. Abramov.
Molecular Neuroscience, Institute of Neurology, University College London, London, United Kingdom.

Frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17) is a neurodegenerative disorder caused by mutations in the *MAPT* gene encoding tau protein. Mitochondrial alterations have been associated with neuronal death in several diseases. The objective of our study was to analyse the mitochondrial function in human iPS cells from a patient of FTDP-17 carrying the 10+16 *MAPT* mutation. In addition, we have selected three different time points of the differentiation from pluripotent stem cells to cortical neurons to study how mitochondrial alterations develop.

We have used fluorescence imaging techniques to examine the mitochondrial function: TMRM to measure the mitochondrial membrane potential ($\Delta\psi_m$) and dyhidroethidium (DHE) to measure the rate of reactive oxygen species (ROS) production.

$\Delta\psi_m$ was higher in iPS-derived neurons from the patient bearing the *MAPT* mutation (158.3 ± 30.2% of control). Higher $\Delta\psi_m$ was also found in non-differentiated pluripotent stem cells (133.4 ± 10.1%) and in the neural rosettes, which represent an earlier stage of the differentiation (151.5 ± 12.4%). In contrast, mitochondrial mass was lower in mutant iPS-derived neurons (85.1 ± 3.9%), although it was similar in non-differentiated cells.

We have also found that the rate of ROS production, measured using DHE, was also higher in iPS-derived neurons from the patient (127 ± 13.9% of control). The increased rate of ROS production in these cells may be the consequence of the enhanced membrane potential. Consistently, the rate of ROS production in non-differentiated cells and in neural rosettes was also significantly higher (123 ± 12.9% and 130 ± 6.9%, respectively).

Our study indicates that this *MAPT* mutation leads to a higher mitochondrial membrane potential, which induces a higher ROS production that may be a trigger for neurodegeneration.

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**The Overexpression of Superoxide Dismutase 1 Restores Growth Defect in a Porin1-Less Yeast Strain and Improves Mitochondrial Metabolism**

**Andrea Magrì**[1], Simona Reina[1], Flora M. Tomasello[2], Maria C. Di Rosa[1], Angela Messina[1], Vito De Pinto[1].
[1]Biological, Geological and Environmental Sciences - Section of Biochemistry and Molecular Biology, University of Catania, Catania, Italy, [2]CNR – Institute of Biostructure and Bioimaging, Section of Catania, Catania, Italy.

Metabolic exchanges between cytosol and mitochondria are made possible by the presence of the pore-forming protein VDAC1 on the outer mitochondrial membrane [1-3]. VDAC1 is directly involved in ATP/ADP, glucose and ions transportation, calcium homeostasis and apoptosis regulation. Moreover, it shows high level of sequence conservation in all eukaryotes: the homologous por1 in yeast S. cerevisiae shows 70% of identity and similar functional properties [1]. Recent studies have highlighted the existence of a link between VDAC1 and SOD1 enzyme, the most important cytosolic defense against superoxide anion. In yeast, SOD1 is required to protect VDAC1 from oxidation but also from carbonylation induced by ROS [3]; in addition, yeast strains devoid of endogenous SOD1 show down-regulated VDAC1 and TOM40 levels, and VDAC shows a significantly less pronounced voltage dependence and conductance [4]. To unravel SOD1 metabolic role in relation to VDAC1-mediated metabolism, we expressed human SOD1 in yeast devoid of endogenous VDAC (Δpor1). While Δpor1 strain cannot grow in the presence of a not-fermentable carbon source, possibly due to altered mitochondria, our results indicates that the overexpression of hSOD1 in Δpor1 strain relieves the growth defect, suggesting that SOD1 participates in the mitochondrial metabolic intersection with the cytosol.

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**3083-Pos Board B513**

**The Role of Complex I in Mitochondrial Reactive Oxygen Species Formation in Cochlear Sensory and Supporting Cells during Ototoxic Aminoglycoside Exposure**

**Danielle Desa**[1], Michael G. Nichols[1], Heather Jensen Smith[2].
[1]Physics, Creighton Univeristy, Omaha, NE, USA, [2]Biomedical Sciences, Creighton Univeristy, Omaha, NE, USA.

Aminoglycosides (AGs) are the most widely used class of antibiotics in the world despite causing permanent hearing loss by damaging inner ear sensory cells. Although the mechanisms of cochlear sensory cell damage are not fully known, reactive oxygen species (ROS) are clearly involved. During normal mitochondrial metabolism low levels of ROS, primarily superoxide, are produced at complexes I and III in the electron transport chain. These levels can increase when mitochondrial dysfunction occurs. Complex I-specific ROS formation was evaluated in acutely-cultured murine cochlear explants exposed to gentamicin (GM, 300 μg/ml), a representative ototoxic AG antibiotic. Mitochondrial membrane potential and pro-apoptotic signaling were measured using Tetramethylrhodamine and apoptosis-inducing factor (AIF) labeling, respectively. Fluorescence intensity-based measurements of nicotinamide adenine dinucleotide (NADH) were used to detect changes mitochondrial metabolism. Relative amounts of superoxide and hydrogen peroxide produced during acute GM exposure were measured using MitoSox Red and Dihydrorhodamine 123, respectively. GM increased NADH fluorescence intensity in low- and high-frequency sensory cells. The complex I inhibitor rotenone (250 nM) significantly increased superoxide, not hydrogen peroxide, in low- and high-frequency sensory cells ($p < 0.01$). GM significantly increased superoxide and hydrogen peroxide formation in low- and high-frequency sensory cells ($p < 0.05$). Rotenone increased GM-induced superoxide formation but decreased GM-induced hydrogen peroxide formation. This effect was greatest in high-frequency cells indicating fundamental differences in ROS formation in high- and low-frequency sensory cells exposed to ototoxic antibiotics. This project provides a base for understanding the underlying mechanisms of mitochondrial ROS production in cochlear cells during exposures to ototoxic antibiotics.

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**Mitochondrial Iron and Sphingosine Synergize Initiation of Hepatocyte Death by Augmenting Oxidative Stress**

**Sergei A. Novgorodov**[1], Tatyana I. Gudz[1], Andaleb Kholmukhamedov[2], Raymond Deepe[2], John J. Lemasters[3,4].
[1]Neurosciences, Medical University of South Carolina, Charleston, SC, USA, [2]Drug Discovery & Biomedical Sciences, Medical University of South Carolina, Charleston, SC, USA, [3]Biochemistry & Molecular Biology, Medical University of South Carolina, Charleston, SC, USA, [4]Institute of Theoretical & Experimentla Biophysics, Pushchino, Russian Federation.

Hepatocytes exposed to ischemia/reperfusion (I/R) succumb to cell death after reperfusion. Sphingosine and ceramide profiles revealed substantial accumulation of sphingosine after 4h of ischemia to rat hepatocytes, whereas other sphingoid bases did not change. A lysosomotropic inhibitor of acid ceramidase suppressed I/R-induced death, indicating a lysosomal origin of sphingosine. Addition of exogenous sphingosine to hepatocytes increased cell death, which was insensitive to the ceramide synthase inhibitor, fumonisin B1. This finding indicates that accumulation of sphingosine, not ceramide formed from sphingosine, promoted cell death. Exogenous sphingosine also inhibited complex IV (cytochrome oxidase), the terminal component of the respiratory chain, in