### S10.28 Differences in $H_2O_2$ production, $\Delta\Psi m$, $JO_2$, electron transport chain enzyme activities and glutamate release in synaptosomes isolated from 6- and 18-month old rats

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Detrimental changes to mitochondrial function have been shown to occur with age. The free radical theory of aging attributes the aquired dysfunction to the stress caused by increased levels of ROS production. In this study we examined the levels of $H_2O_2$ production in synaptosomes and identified markers of age related damage to nerve terminal mitochondrial function, including $\Delta\psi_m$, $JO_2$ and electron trasport chain enzyme complex activities in rats of two age groups, 6 and 18 months old. The rate of $H_2O_2$ production in synaptosomes was found to be higher in the 18-month old group compared to that of the 6-month old group, but were not different in isolated nonsynaptic mitochondria from the two groups. $\Delta\psi_m$ and $JO_2$ were found to be significantly lower in synaptosomes obtained from the brains of 18 month old rats. Measurement of the individual electron trasport chain complex enzyme activities revealed reduced complex II/III and complex IV activities. In addition, $Ca^{2+}$-independent glutamate release was found to be increased at a lower threshold level of complex I inhibition in the older synaptosomes, suggesting an increased susceptibility to low concentrations of inhibitor (rotenone). These data suggest that aging leads to increased nerve terminal reactive oxygen species production while simultaneous deleterious effects occur on the bioenergetic capabilities of *in situ* synaptosomal mitochondria.

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### S10.29 Regulation of $H_2O_2$ generation by uncoupling protein UCP1 in bat and thymus mitochondria

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Teleologically, activated uncoupling proteins (UCPs) would be expected to decrease reactive oxygen species (ROS) generation by the mitochondrial respiratory chain. In our work we applied an Amplex Red/$H_2O_2$ assay to test the ability of UCP1 to regulate ROS generation in rat mitochondria isolated from brown adipose tissue and thymus. Our data show that inhibition of UCP1 by GDP caused a significant increase in ROS generation by brown adipose tissue (BAT) mitochondria. This effect was most apparent in mitochondria respiring on succinate under state 2 conditions (in the absence of rotenone which would allow reverse electron transport), where UCP1 inhibition by GDP caused a ~26 fold ($p<0.01$) increase in ROS generation. In the presence of rotenone the increase was still significant but was reduced to 4.6 fold. In parallel with $H_2O_2$ generation measurements, membrane potential was monitored by uptake of fluorescent probe Safranine. We are currently performing equivalent experiments using BAT mitochondria isolated from UCP1($^{-/-}$) mice and their control littermates to establish whether we can confirm our observation in rat mitochondria and thus whether UCP1 activity could influence ROS production in brown adipose tissue.

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## (S11) Terminal oxidases symposium lecture abstracts

### S11/1 The proton pumping machinery of cytochrome *c* oxidase

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Cytochrome *c* oxidase (Cyt*c*O) is a membrane-bound enzyme, which catalyzes the four-electron reduction of $O_2$ to $H_2O$ and energetically couples this reaction to proton pumping across the membrane. When addressing the proton-pumping mechanism of Cyt*c*O it is particularly constructive to investigate structural variants of Cyt*c*O in which $O_2$ is reduced to water, but the catalytic reaction is uncoupled from proton pumping. There are two classes of such mutant Cyt*c*Os, (*i*) in which the catalytic turnover rate is dramatically slowed due to impaired proton uptake, and (*ii*) in which it is similar to that of the wild-type Cyt*c*O. While in case (*i*) the uncoupling could be conveniently explained in terms of delayed protonation of a "pump site" due to the slowed proton uptake, in the latter case the reason for uncoupling is related to changes in intrinsic thermodynamic/kinetic parameters associated with specific reaction steps within the enzyme. Rationalizing these changes at a molecular level offers mechanistic insights into the structural elements involved in the pumping machinery of Cyt*c*O. In my talk I will present results from studies of several class (*ii*) mutant Cyt*c*O and discuss the molecular mechanism of proton pumping.

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### S11/2 Biogenesis of cytochrome *c* oxidase — bacterial approaches to study cofactor insertion into subunit I

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Biogenesis of cytochrome oxidase is a complex process involving more than 30 known accessory proteins in yeast. Here, we focus on the process of cofactor insertion into subunit I of cytochrome *c* oxidase, using the soil bacterium *Paracoccus denitrificans* as a model organism. CtaG, the *Paracoccus* homolog of yeast Cox11 with a presumed role in copper delivery to the $Cu_B$ center, was purified and characterized spectroscopically. A previously unreported signal at 358 nm allows monitoring copper transfer from copper-loaded CtaG to an acceptor. To mimic a potential cotranslational insertion process, a cell-free expression system has been established, producing subunit I in good yield in the presence of detergents. With such an "open" system it will be feasible to trap and purify assembly intermediates after directly adding individual cofactors, purified assembly proteins, or *P. denitrificans* membranes. Homologous gene loci specifying Surf1 have been identified in *Paracoccus*, and located in two operons for terminal oxidases: *surf*1q is the last gene of the *qox* operon (coding for a $ba_3$-type ubiquinol oxidase), and *surf*1c is found at the end of the *cta* operon (encoding subunits of the $aa_3$-type cytochrome oxidase). Using single and double deletion strains for both *surf*1 genes, we show that both copies are functional, but strictly serve their cognate oxidases only. Cytochrome *c* oxidase was purified from double deletion strain membranes, and the loss of heme $a_3$ in the active site indicates that Surf1c, though not indispensable for oxidase assembly, is involved in an early step of cofactor insertion into subunit I.

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