S12.49 A yeast model of the neurogenic ataxia retinitis pigmentosa (NARP) T8993C mutation in the mitochondrial ATP synthase-6 gene

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Point mutations of the mitochondrially encoded Atp6p subunit of the ATP synthase have been associated with the NARP (neuropathy, ataxia and retinitis pigmentosa) and MILS (maternally inherited Leigh's syndrome) diseases. We report here the construction and properties of a yeast model of one of these mutations, T8993C. This mutation converts the highly conserved leucine 156 of human Atp6p into proline (183 in yeast). The "yeast NARP T8993C mutant" had a good growth on non-fermentable substrates. However *in vitro* the mutant mitochondria showed a 50% decrease in the rates of ATP synthesis and oxygen consumption (at state 3). The slowing down in respiration correlated with a lesser accumulation in complex IV. BN-PAGE analyses revealed a nearly wild type (>90%) content in fully assembled ATP synthase in the mutant. However, low amounts of assembly intermediates (among which the Atp9p- or subunit *c*-ring) were clearly detected also. On intact mitochondria, no significant difference in ATP driven proton translocation was found between the wild type and the mutant. However under condition of maximal ATP hydrolytic activity (non osmotically protected mitochondria at pH 8.4), the mutant activity was two times less efficiently inhibited by oligomycin compared to the wild type, indicating a partial loss in the functional coupling between the F1 and F0 sectors of the ATP synthase.

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S12.50 Intragenic suppressors of the mtDNA T8993G mutation responsible for neuropathy, ataxia, retinis pigmentosa disease, modelled in *Saccharomyces cerevisiae*

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The NARP (neuropathy, ataxia, retinis pigmentosa) disorder has been associated with point mutations of the mitochondrial DNA in the gene encoding the Atp6p subunit of the ATP synthase. The most common and studied of these mutations is T8993G, converting the highly conserved leucine 156 of Atp6p into arginine. We have previously introduced this mutation at the corresponding position (183) of yeast *Saccharomyces cerevisiae* mitochondrially encoded Atp6p. The yeast NARP mutant grows very slowly on respiratory substrates due to a major decrease (>90%) in the rate of mitochondrial ATP synthesis. In the continuity of this study, genetic suppressors improving the respiratory growth of the yeast NARP mutant have been searched. Revertants were isolated from a diploid NARP strain thus favoring the selection of dominant suppressors. They appeared at the rate of 10^{-5}. All were found to be intragenic revertants, i.e. issued from a second mutation in the ATP6 gene. Seven different intragenic suppressors were identified at either the original mutated codon (first-site suppressors) or in another position of Atp6p (second-site suppressors). Mitochondria were prepared from the revertants and their energy transducing activities characterized. The results indicate that the T8993G mutation is responsible for a local volumic constraint rather than a charge hindrance. Importantly, it appears that discrete changes in specific regions of Atp6p structure can compensate efficiently for the presence of the pathogenic leucine to arginine change.

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(S13) Electron transport chain and proton pumps symposium lecture abstracts

S13/1 Heme plus apoprotein = c-type cytochrome: Not so simple

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c-type cytochromes encompass a variety of proteins, from the well known monoheme cytochromes *c* and c_1 of the mitochondrial and some bacterial electron transfer systems, to the multiheme proteins in a wide range of bacterial respiratory processes, for example reduction of sulfate. The common feature of (almost) all these proteins is that their heme moiety is attached via two thioether bonds to the two cysteines of a CXXCH motif, where the histidine is usually an axial ligand to the heme iron. There are exceptions where, for example, there is one cysteine in the motif (trypanosome mitochondria). Attachment of heme to the two cysteines requires a post-translational apparatus. Surprisingly, there are at least four types of distinct apparatus for this process, and even within one type there appear to be significant variations. Features of the most complex, System I (bacteria and plant mitochondria), will be reviewed and an overview given of Systems II (chloroplasts and bacteria) and III (mitochondria from many species). The curious absence of a recognisable system in trypanosomes and related eukarytotes will also be discussed.

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S13/2 Conformational changes and activity of complex II

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The aim of this study was to determine the role of an 11-amino acid loop that is part of the capping domain in the flavoprotein subunit of the complex II homologues succinate:ubiquinone oxidoreductase (SQR) and quinol:fumarate reductase (QFR). To investigate the role of the conserved loop in the interaction of complex II with dicarboxylate substrates and inhibitors two threonine residues were altered by site-directed mutagenesis. Threonine-234 in the flavoprotein subunit (FrdA) of QFR (equivalent to Thr244 of the flavoprotein subunit of SQR, SdhA) is part of the hinge region connecting the flavin and capping domains of QFR and SQR, respectively. FrdA Thr234 and SdhA Thr244 were mutated to Ala residues and the catalytic properties of the resulting enzymes were investigated. These investigations included a combination of kinetics, optical spectral analysis, and for the FrdA Thr234Ala mutant X-ray crystallography. An additional threonine residue in QFR and SQR which is conserved in the loop was also investigated. FrdA Thr244/SdhA Thr254, which is hydrogen-bonded to the C1 of the dicarboxylate substrate, was also substituted with