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

Roza Kucharczyk, Jean-Paul di Rago
Institut de Biochimie et Génétique Cellulaires du CNRS, Université Bordeaux2, Bordeaux, France
E-mail: roza@ibb.waw.pl

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*

Maïlis Bietenhader[1], Roza Kucharczyk[1], Malgorzata Rak[1], Nahia Ezkurdia, Jean-Paul di Rago
Institut de Biochimie et Génétique Cellulaire, CNRS-Université Bordeaux2, Bordeaux, France
E-mail: mailis.bietenhader@ibgc.cnrs.fr
[1] These authors contributed equally.

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

Stuart J. Ferguson
Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, UK

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

Gary Cecchini[a], Elena Maklashina[a], Thomas M. Tomasiak[b], Tina M. Iverson[b]
[a]*Molecular Biology, VA Medical Center, San Francisco and Department of Biochemistry and Biophysics, University of California, San Francisco, USA*
[b]*Department of Pharmacology and Biochemistry, Vanderbilt University Medical Center, Nashville, Tennessee, USA*
E-mail: Gary.Cecchini@ucsf.edu

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

an alanine residue. It was found that this substitution significantly altered the charge transfer band seen in wild-type QFR and SQR with oxaloacetate. The spectral and kinetic data are consistent with a loss of catalysis showing the importance of the H-bond to substrate in the mechanism of fumarate reduction and succinate oxidation by both SQR and QFR. The X-ray crystallography of the FrdA Thr234Ala enzyme also shows a dramatic domain rearrangement between the capping domain and flavin domain in FrdA. This movement opens a substrate channel to the active site of the enzyme by altering the capping domain position. A comparison of the location of the capping domain in the open and closed states in the mutant protein suggests that in complex II enzymes, movement of the domain may be coupled to stabilization of the transition state by the threonine side chain.

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S13/3 Activated Q-cycle as a common mechanism for the cytochrome bc_1 and cytochrome b_6f complexes

Armen Y. Mulkidjanian[a,b], Sergey S. Klishin[a,b], Natalia E. Voskoboynikova[a]
[a]*University of Osnabrück, Osnabrück, 49069, Germany*
[b]*Moscow State University, Moscow, 119991, Russia*
E-mail: amulkid@uos.de

The aim of our studies is to understand the electron/proton coupling in the cytochrome bc_1 complex (bc_1). The functioning of bc_1 can be described by a dimeric Q-cycle scheme. The main traits of such a mechanistic scheme, which we have been developing during the last decade, are (i) the possibility of electron exchange between the monomers, (ii) the alternating ubiquinol binding in two ubiquinol-oxidizing centers *P*, (iii) the electrostatic compensation of electron transfer up to the ultimate step of the center *N*-catalyzed ubiquinol formation that is coupled with major voltage generation. Based upon own data on the kinetic correlation between the flash-induced redox changes of cytochrome *b*, voltage generation, and proton transfer in membrane vesicles of *Rhodobacter capsulatus*, we have put forward a scheme of a dimeric, activated Q-cycle. This scheme implies that under physiological conditions the bc_1 is maintained in an "activated" state, with a bound semiquinone in center *N* of one monomer and a reduced high-potential heme *b* in the other monomer, owing to continual priming by oxidation of membrane ubiquinol via center *N*. If bc_1 is pre-activated, then, in accordance with experimental observations, oxidation of each ubiquinol molecule in center *P* leads to ubiquinol formation in the one of enzyme's centers *N* and to the voltage generation. The applicability of this scheme to the plant cytochrome b_6f-complexes will be discussed.

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S13/4 Structural and biochemical characterisation of the alternative oxidases

Anthony L. Moore[a], Paul G. Crichton[b], Mary S. Albury[a]
[a]*Biochemistry and Biomedical Sciences, University of Sussex, Falmer, Brighton, UK*
[b]*Current address: MRC Dunn Human Nutrition Unit, Hills Road, Cambridge, UK*
E-mail: a.l.moore@sussex.ac.uk

In addition to the conventional cytochrome *c* oxidase, plant mitochondria contain a nonprotonmotive alternative oxidase (AOX) that couples the oxidation of ubiquinol directly to the reduction of molecular oxygen. In thermogenic plants, AOX is responsible for heat generation, whilst in non-thermogenic species, the oxidase is thought to play a more fundamental role in the regulation of energy metabolism. AOX may be involved in facilitating TCA cycle turnover, protection against oxidative stress, and preservation of plant growth homeostasis. AOX proteins are not restricted to plants, but also occur in pathogenic organisms including the blood parasite *Trypanosoma brucei* and the intestinal parasite *Cryptosporidium parvum*. Because of their absence in the mammalian host, AOX proteins are potential therapeutic targets in these systems. Although no high-resolution AOX structure is available to date the accepted structural model predicts that AOX is an integral (~32 kDa) interfacial membrane protein that interacts with a single leaflet of the lipid bilayer, and contains a non-haem diiron carboxylate active site. This model is supported by extensive site-directed mutagenesis studies and EPR spectroscopic experiments have confirmed the presence of a binuclear iron centre. This talk will focus on the recent identification of other residues and regions important for enzyme catalysis, access of oxygen to the active-site and ubiquinol-binding.

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(S13) Electron transport chain and proton pumps symposium abstracts (poster and raised abstracts)

S13.5 An alternative site for proton entry from the cytoplasm to the quinone binding site in the *Escherichia coli* succinate dehydrogenase

Victor W.T. Cheng, Antonia Johnson, Richard A. Rothery, Joel H. Weiner
Department of Biochemistry, University of Alberta, Edmonton, AB, Canada
E-mail: vcheng@ualberta.ca

Escherichia coli succinate dehydrogenase (Sdh) belongs to the highly conserved Complex II family of enzymes which does not generate a proton motive force during catalysis. Because of its electroneutrality, the quinone reduction reaction must consume cytoplasmic protons which are released stoichiometrically from succinate oxidation. The X-ray crystal structure of *E. coli* Sdh shows that residues $SdhB^{G227}$ and $SdhC^{D95}$, as well as $SdhC^{E101}$, are located at or near the entrance of an observed water channel that has been proposed to function as a proton wire connecting the cytoplasm to the quinone binding site. However, the pig and chicken Sdh enzymes show an alternative entrance to the water channel that is greeted by the conserved $SdhD^{Q78}$ residue. In this study, these four residues were studied by site-directed mutagenesis. We show that the observed water channel in the *E. coli* structure is the functional proton wire *in vivo*, while *in vitro* results indicate alternative entrances for protons, possibly located at $SdhD^{Q78}$. *In silico* examination of the *E. coli* Sdh also reveals a possible H-bonding network leading from the cytoplasm to the quinone binding site, also via $SdhD^{Q78}$. Based on these results we propose an alternative proton pathway in *E. coli* Sdh that is functional only *in vitro*.

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S13.6 Heme-free variants of *Escherichia coli* succinate dehydrogenase

Quang M. Tran[a], Richard A. Rothery[a], Elena Maklashina[b,c], Gary Cecchini[b,c], Joel H. Weiner[a]
[a]*Department of Biochemistry, University of Alberta, Edmonton, AB, Canada*
[b]*Molecular Biology Division, Veterans Affairs Medical Center, San Francisco, CA*
[c]*Department of Biochemistry and Biophysics, University of California, San Francisco, CA*
E-mail: qmtran@ualberta.ca