cluster to permit physiological electron transfer. The unusually large movement could suggest a mechanism for coupling. To gain further insight into the mechanism of complex I, we describe a series of experiments on structural and functional characterisation of the interaction between T. thermophiles complex I and quinone or quinone-like inhibitors.

Reference

doi:10.1016/j.bbabio.2012.06.166

6P27

Low abundance of the matrix arm of complex I of mice liver mitochondria predicts longevity

Satomi Miwa, Howsun Jow, Achim Treumann, Thomas von Zglinicki
Newcastle University, Institute for Ageing and Health, Campus for Ageing and Vitality, Newcastle upon Tyne, NE4 5PL, UK
E-mail: satomi.miwa@ncl.ac.uk

Dietary restriction (DR) extends lifespan in many strains of mice. It is accompanied by the reduction in reactive oxygen species (ROS) release from complex I of the mitochondria from various tissues, and aging is found to increase it. However, the nature of mitochondrial alterations resulting in these observations is not fully understood.

We compared the proteome of purified liver mitochondria using 6plex isobaric labelling, isoelectric focusing and LC-MS/MS from young mice in which lifespan was prolonged by either DR or genetically (long lived strain, ICRFa) and old mice. We developed a Bayesian model that enabled us to detect small differences in protein abundance with high confidence. DR induced a number of specific changes in liver mitochondria including enrichment of proteins involved in fatty acid metabolism, amino acid degradation and TCA cycle. Importantly, the abundance of specific components of the electron transport chain and of prohibitins 1 and 2 were similarly decreased in all young mice with enhanced longevity, and many of them were enriched in old mice. Most interestingly, the changes within complex I were highly clustered: We quantified 31 out of the 45 known protein subunits of complex I. None of the subunits belonging to the membrane hydrophilic arm were significantly changed among the groups, but 14 out of the 17 identified proteins forming the matrix arm of complex I, where all the catalytic centres are located, were downregulated under DR; many of them were also downregulated in genetically long lived mice but became enriched in old. Blue native electrophoresis confirmed that DR decreased the abundance of partially assembled complex I sub-complexes.

Our observations suggest the intriguing possibility that a balanced composition of complex I and its proper assembly might regulate mitochondrial function and thus ageing: higher abundance of complex I catalytic subunits that are not completely assembled would not improve electron transport but might enhance ROS production. This could explain why short-lived and older animals show less efficient oxygen consumption together with increased ROS production from complex I.

6P28

ROS-production by E. coli respiratory complex I

Klaudia Morina, Marius Schulte, Sascha Jurkovic, Sabrina Burschel, Oliver Einsle, Thorsten Friedrich

Institut für Organische Chemie und Biochemie, Albert-Ludwigs-Universität, Albertstr. 21, D–79104 Freiburg, Germany
E-mail: klaudia.morina@ocbc.uni-freiburg.de

The respiratory complex I couples the electron transfer from NADH to ubiquinone with a proton translocation across the membrane. The bacterial complex consists of 13 to 15 subunits and harbors one FMN and seven to ten Fe/S-clusters depending on the species. The X-ray structure of the complex revealed that the NADH binding site is made up by a novel type of Rossman-fold providing binding sites for FMN and NADH [1]. We solved the structure of the NADH binding site of the Aquifex aeolicus complex with bound substrates at 2 Å resolution [2]. We showed that Glu183 on Nuof plays a central role in discriminating NADH and NADPH as substrates. Glu183 was replaced by Gln, Asp, Asn, His, Ala and Gly and the NAD(P)H:decylubiquinone oxidoreductase activities of the isolated variants were measured. All variants showed an increased catalytic efficiency with NADPH and NADH. All variants showed lower ROS production with NADPH compared to the wildtype. The NADH oxidation is accompanied with an enhanced ROS production most likely due to the mode of nucleotide binding [3]. To correlate the increased ROS production with nucleotide binding, the A. aeolicus variants with bound substrates were crystallized.

References

doi:10.1016/j.bbabio.2012.06.168

6P29

Characterization of the inhibitor-binding site in mitochondrial NADH-ubiquinone oxidoreductase (complex I) using fenpyroximate analogues

M. Murai, Y. Shiraishi, N. Sakiyama, H. Miyoshi
Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Sakyoo-ku, Kyoto 606-8502, Japan
E-mail: m_murai@kais.kyoto-u.ac.jp

Fenpyroximate, a strong inhibitor of bovine NADH-ubiquinone oxidoreductase (complex I), had been reported to bind to the ND5 subunit [1]. Considering that the main body of the NDS subunit is located at the distal end of the membrane domain as a part of protonpumping module [2], however, the previous result may be questionable. Because establishing the number and location of inhibitors and/or quinone binding sites in the membrane domain is necessary to elucidate the function of the enzyme, it is critical to clarify whether there are additional inhibitors and/or quinone binding site besides the interface between the hydrophilic and membrane domains. We therefore performed the photolabeling experiments using two newly synthesized fenpyroximate derivatives [125I]APF and [125I]AIF, possessing a photoreactive azido group at and far from the pharmacophoric core moiety, respectively. Extensive biochemical and proteomic analysis of the labeled bovine complex I revealed that the residues labeled by [125I]APF and [125I]AIF are located in the region Ser43-Arg66 (PSST) and Asp160-Arg174 (49 kDa), respectively [3], which face the supposed quinone-binding pocket formed at the interface of the PSST, 49 kDa, and ND1 subunits. These results