chain. The 14 core subunits of the complex are conserved from prokaryotes through to higher eukaryotes. The prokaryotic complex lacks the ~30 subunits that are found in the mitochondrial enzyme, making it an attractive model by which to probe the structure and mechanism. Recently, the crystal structure of complex I from *Thermus thermophilus* has been determined to a resolution of 3.3 Å. It suggests that the proton translocation is driven by co-ordinated conformational changes [2]. Due to the established crystallisation methods and the genetic toolbox available for *T. thermophilus*, this species was chosen to be a model in which to probe the mechanism of proton pumping, by site-directed mutagenesis. The systems by which genetic manipulation can be carried out in *T. thermophilus* have been established and used to previously knock out a variety of genes. When applied to complex I subunits however, no chromosomal gene knock outs have been obtained. When screening for mutants using PCR it was consistently found that both the wild type (WT) copy of the gene and the introduced knock out cassette are found within the cell, which displays a WT phenotype. Some controversy in the literature exists as to whether *T. thermophilus* exists as a monoploid or a polyploid bacterium. Our results appear to be consistent with the view that *T. thermophilus* maintains one copy of its chromosome and that illegitimate recombination is taking place within the 200 kbp megaplasmid that it harbours. In addition to mechanistic mutations the role of two supernumerary subunits [2] that are unique to *T. thermophilus* were probed. The role of these is at present unclear; Nqo15 is speculated to be frataxin-like and may play a role in the assembly of iron–sulphur clusters whereas Nqo16 is postulated to be an assembly factor for the complex. An antisense RNA knockdown approach was used to probe the role of these subunits, the results of which are discussed.

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doi:10.1016/j.bbabio.2014.05.051

S4.P4

Unraveling the coupling mechanism of complex I and the role of antiporter-like subunits

Paulo J.G. Castro, Ana P. Batista, Manuela M. Pereira
Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Av. da República EAN, 2780-157 Oeiras, Portugal
E-mail address: paulo.castro@itqb.unl.pt

Respiratory complex I plays a central role in energy production by coupling electron transfer between NADH and quinone to ion translocation across the membrane, thereby establishing an electrochemical potential. This L-shaped enzyme consists of hydrophilic and membrane domains. The membrane domain includes seven hydrophobic subunits and the three largest subunits, NuoL, M and N (*Escherichia coli* nomenclature), are homologous to each other and to Na^+/H^+ antiporter complex (Mrp) subunits [1,2]. Previous studies indicate that complex I from *Rhodothermus marinus* transduces energy by two different processes: proton pumping and Na^+/H^+ antiporting [3]. This work aims at evaluating the ability of the isolated complex I to translocate H^+ and Na^+ across the membrane and to determine the stoichiometry of the process. Moreover, we intend to evaluate the role of the individual membrane antiporter-like subunits. In order to achieve our goal, the enzyme was purified from *R. marinus* and incorporated into liposomes. The proteoliposomes were characterized by Dynamic Light Scattering (DLS). The existence of NADH:quinone oxidoreductase activity and the formation of membrane potential after addiction of the substrates proved that the incorporation was successful. H^+ and Na^+ translocation were monitored by fluorescence spectroscopy and ^{23}Na NMR, respectively. The antiporter-like subunits of complex I (NuoL, NuoM, NuoN) are composed of two structural inverted repeat structures. Each of these subunits and in the case of NuoL, each individual inverted repeat, were cloned and expressed together with a Maltose binding protein (MBP). The role of the individual subunits, namely in ion transport is investigated.

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doi:10.1016/j.bbabio.2014.05.052

S4.P5

Role of LdcI for the assembly of the *Escherichia coli* complex I

Doris Kreuzer Dekovic[a], Heiko Erhardt[a],
Stefan Steimle[a], Thorsten Friedrich[b]
[a]*Institute of Biochemistry, Albert Ludwigs University Freiburg, Germany*
[b]*Albert-Ludwigs-Universitat Freiburg, Germany*
E-mail address: doris.dekovic@sgbm.uni-freiburg.de

NADH:ubiquinone oxidoreductase (complex I) is the main electron entry point in the respiratory chains of eukaryotes and bacteria. It couples the transfer of electrons from NADH to ubiquinone with the translocation of protons across the membrane. Complex I of *Escherichia coli* consists of 13 subunits, named NuoA-N, an FMN and 9 iron–sulfur (Fe/S) clusters [1]. Very little is known about the assembly of bacterial complex I, however it is reasonable to assume that the complex is assembled by joining together prefabricated smaller parts. The inducible lysine decarboxylase (LdcI) was identified as a possible assembly factor. Deletion of NuoL resulted in production of two distinct complex I populations, an active and an inactive one. While the active one contained all known cofactors and all subunits except NuoL, the inactive one lacked the most distal Fe/S cluster N2 and was associated with LdcI [2]. To further investigate the interaction between complex I and LdcI in vivo FRET measurements were performed.

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doi:10.1016/j.bbabio.2014.05.053