

energy metabolism and mitochondrial function. Whereas insect stage parasites maintain a well developed single mitochondrion that produces ATP via oxidative and substrate-level phosphorylation, the bloodstream stage generates ATP exclusively via glycolysis. Although the cytochrome-containing respiratory complexes are absent from the inner membrane of the bloodstream stage mitochondrion, other activities, including respiratory complex V (ATP synthase) and an alternative oxidase, are expressed and in fact essential. While the presence or absence of respiratory complexes II–V in the bloodstream stage mitochondrion is firmly established, the existence of mitochondrial complex I in trypanosomes is the subject of a long-standing controversy in the field. Genes encoding putative subunits of complex I can be identified in the *T. brucei* genome [1] and several of the corresponding proteins have been identified in a putative oxidoreductase complex isolated from insect stage parasites [2]. In order to definitively establish whether complex I exists and is functional in bloodstream stage trypanosomes we use a combination of affinity purification strategies and gene knockout studies. We have expressed tagged versions of four putative subunits – NUBM (51 k, Nqo1), NUKM (NdhK, Nqo6), acyl-CoA ligase-like protein (ACSL) and LYR motif protein 4 (LYRM4) – and have demonstrated that at least three of these localize to the mitochondrion and that ACSL and LYRM4 comigrate in a possible complex on glycerol gradients. Tag-mediated pulldown of NdhK also pulled down ACSL, corroborating an association between the two molecules. Thus, our current data suggest that at least a partial complex I is assembled in bloodstream form *T. brucei*. Interestingly, we were able to generate null mutants for NUBM and NUKM, indicating that this complex is non-functional as an NADH:ubiquinone oxidoreductase or redundant.

#### References

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doi:10.1016/j.bbabbio.2010.04.086

#### 1P.39 Inhibition of the NADH:ubiquinone oxidoreductase (complex I) by $Zn^{2+}$

Marius T. Schulte<sup>1</sup>, Dinah Mattay<sup>2</sup>, Katerina Dörner<sup>1</sup>, Petra Hellwig<sup>3</sup>, Thorsten Friedrich<sup>1</sup>

<sup>1</sup>Institut für Organische Chemie und Biochemie, Albert-Ludwigs-Universität Freiburg, Germany

<sup>2</sup>BAG Health Care GmbH, Germany

<sup>3</sup>Institut de Chimie, Laboratoire d'électrochimie Strasbourg, France

E-mail: Marius-in-Freiburg@web.de

The energy-converting NADH:ubiquinone oxidoreductase (complex I) couples the transfer of electrons from NADH to ubiquinone with the translocation of protons across the membrane. It was shown that  $Zn^{2+}$  inhibits proton translocation of many proton-translocating membrane proteins. We studied the effect of  $Zn^{2+}$  on electron transfer and proton translocation by the *E. coli* complex I and the NADH-dehydrogenase fragment of the complex. It turned out that  $Zn^{2+}$  inhibited both activities of complex I in a pH-dependent manner. The electrontransfer of the NADH dehydrogenase fragment was also inhibited but at a lower  $IC_{50}$ . This indicates that complex I has at least two  $Zn^{2+}$  binding sites. Complex I was not inhibited by other mono- or bivalent cations except  $Ag^+$  [1], which is expected to react with the flavin mononucleotide [2]. The most distal iron–sulfur cluster N2 [3], expected to be involved in quinone binding, was only partially reduced in the presence of  $Zn^{2+}$ . As  $Zn^{2+}$  is expected to block proton translocation this finding is the first experimental evidence for a conformational change of the surrounding of cluster N2 due to proton translocation.

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doi:10.1016/j.bbabbio.2010.04.087

#### 1P.40 Assembly of the *Escherichia coli* NADH:ubiquinone oxidoreductase (complex I)

Stefan Steimle, Thomas Pohl, Thorsten Friedrich

Albert-Ludwigs-Universität Freiburg, Institut für Organische Chemie und Biochemie, Germany

E-mail: stefansteimle@web.de

The energy-converting NADH:ubiquinone oxidoreductase, the respiratory complex I, of *Escherichia coli* consists of 13 subunits named NuoA – NuoN [1]. We used *E. coli* strains in which the *nuc*-genes, coding for the complex I subunits, are individually disrupted by insertion of a resistance cartridge to study the assembly of the complex in the mutants [2]. No complex I specific activity was detected in the membranes of the mutants. However, the cytoplasmic fraction of some of the mutants contained the fully assembled NADH dehydrogenase fragment of the complex. In addition, a partially assembled complex I was detected in the membranes of the *nucL* mutant. For characterization of this fragment all *nuc*-genes but *nucL* were overexpressed using the system established in our lab [3]. The overproduced complex I variant was isolated from the mutant. Two populations were obtained. In both populations the subunit NuoL was missing. One population showed no activity and was lacking Fe/S cluster N2. This preparation was associated with a *bona fide* chaperone. The other population contained all Fe/S clusters of complex I. It showed about two thirds of the electron transfer activity of the wild type complex I. After reconstitution in proteoliposomes this preparation showed a proton translocation activity which was approximately half of that of the wild type complex I.

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doi:10.1016/j.bbabbio.2010.04.088

#### 1P.41 Statistical analysis of experimental data on titration of metal centers in respiratory complex I

Emile S. Medvedev<sup>1</sup>, Vernon A. Couch<sup>2</sup>, Alexei A. Stuchebrukhov<sup>2</sup>

<sup>1</sup>Institute of Problems of Chemical Physics, Russian Academy of Sciences, 142432 Chernogolovka, Russia

<sup>2</sup>Department of Chemistry, University of California, Davis, CA 95616, USA

E-mail: stuchebr@chem.ucdavis.edu

Recently, Euro et al. (Biochem., 2008, 47: 3185) have reported titration data for seven of nine FeS redox centers of complex I from *E. coli*. There is a significant uncertainty in the assignment of the data. Four of the titration curves were assigned to N1a, N1b, N6b, and N2; one curve either to N3 or N7; one more either to N4 or N5; and the last one denoted Nx could not be assigned at all. In addition, the assignment of the N6b signal is also uncertain, and the signal might belong to N6a. In this paper, using our calculated interaction energies (Couch et al., 2009, Biochim. Biophys. Acta 1787: 1266), we perform statistical analysis of these data and determine the intrinsic redox potentials of the centers; out of 24 possible assignments of the data we find the best fit, and a few less