Complex I is the proton pumping NADH:ubiquinone oxidoreductase of the mitochondrial inner membrane. Complex I from bovine mitochondria contains eight iron–sulphur clusters (two [2Fe–2S] clusters and six [4Fe–4S] clusters). Seven of them link the NADH oxidation site to the ubiquinone reduction site. The eighth cluster, named 2Fe[24] or N1a, is ligated by the 24 kDa subunit; it is isolated from the main chain of clusters but adjacent to the flavin mononucleotide and close enough to accept electrons from it. Whether the 2Fe[24] cluster has a role in the mechanism of complex I is not known. It is possible it minimises the lifetime of the semi-reduced flavin species, decreasing the rate of superoxide production and/or preventing direct hydrogen peroxide production by the fully reduced flavin. Complex I from Escherichia coli contains a homologous cluster with a reduction potential 0.1 V higher than that of the bovine cluster; complex I from E. coli also produces hydrogen peroxide rather than superoxide. The E. coli cluster is probably reduced during catalytic turnover, and may be incapable of minimising the semi-reduced flavin. In this study, complex I from Yarrowia lipolytica was used to establish the role of the 2Fe[24] cluster. Mutations were generated in the closely homologous NUMH subunit, to increase the reduction potential of the [2Fe–2S] cluster to that observed in E. coli. The effects on catalysis and superoxide production by the complex are described.

doi:10.1016/j.bbabio.2010.04.052

1P.6 The role of the isolated [2Fe–25] cluster adjacent to the flavin mononucleotide of mitochondrial complex I: Does it influence catalysis at the flavin site?
James A. Birrell, Judy Hirst
Medical Research Council, Mitochondrial Biology Unit, Cambridge, UK
E-mail: jb@mrc-mbu.cam.ac.uk

Complex I is the proton pumping NADH:ubiquinone oxidoreductase of the mitochondrial inner membrane. Complex I from bovine mitochondria contains eight iron–sulphur clusters (two [2Fe–2S] clusters and six [4Fe–4S] clusters). Seven of them link the NADH oxidation site to the ubiquinone reduction site. The eighth cluster, named 2Fe[24] or N1a, is ligated by the 24 kDa subunit; it is isolated from the main chain of clusters but adjacent to the flavin mononucleotide and close enough to accept electrons from it. Whether the 2Fe[24] cluster has a role in the mechanism of complex I is not known. It is possible it minimises the lifetime of the semi-reduced flavin species, decreasing the rate of superoxide production and/or preventing direct hydrogen peroxide production by the fully reduced flavin. Complex I from Escherichia coli contains a homologous cluster with a reduction potential 0.1 V higher than that of the bovine cluster; complex I from E. coli also produces hydrogen peroxide rather than superoxide. The E. coli cluster is probably reduced during catalytic turnover, and may be incapable of minimising the semi-reduced flavin. In this study, complex I from Yarrowia lipolytica was used to establish the role of the 2Fe[24] cluster. Mutations were generated in the closely homologous NUMH subunit, to increase the reduction potential of the [2Fe–2S] cluster to that observed in E. coli. The effects on catalysis and superoxide production by the complex are described.

doi:10.1016/j.bbabio.2010.04.054

1P.7 Mitochondrial acyl carrier proteins in Yarrowia lipolytica: Guilty by affiliation with complex I
Martina Ding, Ulrich Brandt
Molecular Bioenergetics Group, Medical School, Cluster of Excellence Frankfurt “Macromolecular Complexes”, Center for Membrane Proteomics, Goethe University, Frankfurt am Main, Germany
E-mail: Ding@zbc.kgu.de

Mitochondrial acyl carrier proteins (ACPMs) were first discovered in the 1980s in Neurospora crassa. They are thought to be involved in mitochondrial fatty acid synthesis and in the production of octanoic acid via a phosphopantetheine group covalently attached to a conserved serine. Our group has previously demonstrated that Yarrowia lipolytica codes for two different mitochondrial acyl carrier proteins, ACPM1 and ACPM2, that both are bona fide subunits of complex I. Deletion of the ACPM1 gene is lethal, whereas ACPM2Δ strains are viable in a certain strain background. However, the ACPM2Δ cells showed an apparent lack of complex I, pointing towards a role in assembly/stability for the complex. In contrast, ACPM1 seems to have a function beyond complex I. The two ACPM protein sequences differ mostly in their putative mitochondrial targeting sequences. We thus created a protein consisting of the ACPM1 targeting sequence fused to the sequence of mature ACPM2. Two DNA constructs with different length of the putative ACPM1 targeting sequence were created and used for plasmid-based complementation of the ACPM1Δ strain. No viable spores were obtained, indicating that both chimeric proteins failed to take over the function of ACPM1. In the ACPM2Δ strain, both constructs led to the formation of assembled complex I, suggesting that the functional difference between the ACPM variants is mediated by the targeting sequence. Currently, various domain-swap constructs are underway

doi:10.1016/j.bbabio.2010.04.053