small copper protein called sulfocyanin. This complex is enzymatically functional, reducing oxygen in the presence of the electron donor ferrous iron, at low pH. A second putative cytochrome ba-Rieske protein complex, reminiscent of archaeal complexes analogous to the bc1 complex, may be involved in regeneration of reducing equivalents by reverse electron flow. A model of energy ferrous iron metabolism of *F. acidiphilum* is proposed [1]. This study constitutes the first detailed biochemical investigation of iron metabolism in acidophilic Archaea, and confirms that iron respiratory chains are clearly different from an organism to another even among Archaea.

Reference

[1] C.J. Castelle, M. Roger, M. Bauzan, M. Brugna, S. Lignon, M. Nimtz, O. Golyshina, M.-T. Giudici-Orticoni, M. Guiral, New insight into the aerobic respiratory chain of the iron-oxidizing archaeon *Ferroplasma acidiphilum* living in extreme acidic environments, submitted (2014).

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S8.P14

Structure and function of the Rnf complex from *Acetobacterium woodii*

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The anaerobic acetogenic bacterium *Acetobacterium woodii* has a novel Na^+-translocating electron transport chain that couples electron transfer from reduced ferredoxin to NAD^+ with the generation of a primary electrochemical Na^+ potential across its cytoplasmic membrane [1]. The enzyme was identified to be encoded by the rnf genes previously identified to be essential for nitrogenase function in *Rhodobacter* [2]. The Rnf genes are widely distributed in Bacteria, indicating that this novel ion-translocating electron transport chain is widely distributed [3]. In most anaerobic bacteria, the Rnf complex functions in ferredoxin-driven NAD^+ reduction coupled to the generation of a transmembrane ion gradient, but we will show here that it also catalyzes the reverse function in vivo, ferredoxin reduction with NADH as reductant at the expense of the electrochemical sodium ion gradient. This reaction is important for many anaerobes, but also the most likely in vivo function for aerobes or facultative aerobes in which the Rnf complex provides reduced ferredoxin for biosynthetic (N_2 fixation) or regulatory functions. The Rnf complex is encoded by six genes whose products are involved in NAD^+ binding, binding of reduced ferredoxin, electron flow from reduced ferredoxin to NAD^+ and coupling of the electron transfer reaction to Na^+ transport but the function of the individual subunits remains elusive. Here, we present a structure-function analysis of single subunits of the Rnf complex of *A. woodii*.

References

[1] E. Biegel, V. Müller, Bacterial Na^+-translocating ferredoxin:NAD^+ oxidoreductase, Proc. Natl. Acad. Sci. U.S.A. 107 (2010) 18138–18142.
[2] M. Schmehl, A. Jahn, A. Meyer zu Vilsendorf, S. Hennecke, B. Masepohl, M. Schuppler, M. Marxer, J. Oelze, W. Klipp, Identification of a new class of nitrogen fixation genes in *Rhodobacter capsulatus*: a putative membrane complex involved in electron transport to nitrogenase, Mol. Gen. Genet. 241 (1993) 602–615.
[3] E. Biegel, S. Schmidt, J. M. González, V. Müller, Biochemistry, evolution and physiological function of the Rnf complex, a novel ion-motive electron transport complex in prokaryotes, Cell. Mol. Life Sci. 68 (2011) 613–634.

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S8.P15

Respiratory type II NAD(P)H dehydrogenase of *Zymomonas mobilis* with altered cofactor specificity

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NADH type II dehydrogenase (Ndh) in bacteria oxidizes NAD(P)H, without contributing to the generation of protonmotive force. For the facultatively anaerobic ethanol-producing bacterium *Zymomonas mobilis* this is the sole functional respiratory NAD(P)H dehydrogenase, able to oxidize both cofactors, although with a preference for NADH. Rapid, yet energetically uncoupled respiratory chain of this bacterium might be useful for biotechnological application in bioconversions, where redox balancing is needed, but synthesis of excess ATP is unwanted. However, the redox cofactor specificity is important for such applications. *Z. mobilis* is a perspective producer of bioethanol. Its both alcohol dehydrogenase (ADH) isoenzymes are NAD(H)-specific. If novel substrate pathways would involve generation of excess reducing equivalents, NADP(H) should preferably be the redox cofactor for recycling, to avoid competition between the respiratory chain and ADH reaction for NADH, leading to accumulation of acetaldehyde and fall of ethanol yield. To increase the NAD(P)H specificity of Ndh, we replaced the two amino acid residues located at the end of the second beta-sheet of the NAD(P)H-binding domain of Ndh by site-directed mutagenesis, as previously demonstrated for *Agrobacterium tumefaciens* [1]. Glutamate in the position 219 of the Ndh protein was replaced by glutamine, and alanine in the position 220 — by serine. Mutated variants of *ndh* (ZMO1113) (including their own promoter regions) were inserted in the shuttle vector pBBR1MCS-2 and transformed into the strain with an Ndh-deficient background (strain Zm6-*ndh*, derived from Zm6 ATCC29191) [2]. The mutant strains showed a decrease of NAD(P)H dehydrogenase activity by more than an order of magnitude, yet, in contrast to the wild type Ndh, Vmax for NADPH exceeded the respective value for NADH. Kinetic properties of the mutant Ndh, and potential implications of these mutations for aerobic growth energetics of the recombinant *Z. mobilis* strains are discussed.

References

[1] C. Desplats, A. Beyly, S. Cuiné, L. Bernard, L. Cournac, G. Peltier, Modification of substrate specificity in single point mutants of *Agrobacterium tumefaciens* type II NADH dehydrogenase, FEBS Lett. 581 (2007) 4017–4022.
[2] U. Kalnenieks, N. Galinina, I. Strazdina, Z. Kravale, J. L. Pickford, R. Rutkis, R. K. Poole, NADH dehydrogenase deficiency results in low respiration rate and improved aerobic growth of *Zymomonas mobilis*, Microbiology 154 (2008) 989–994.

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Phylogenomic analysis of membrane Na^+-translocating decarboxylases

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