14P.9 Antiporter activity of the individual complex I subunits NuoL, NuoM and NuoN from *Escherichia coli* analyzed in an *in vivo* model system

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The complex I (NADH:quinone oxidoreductase) membrane spanning subunits NuoL, M and N are homologous to one particular class of Na^+ or K^+/H^+ antiporters, encoded by the gene cluster denoted *mrp/sha/pha/mnh* in different bacteria. These subunits are prime candidates for harboring important parts of the proton pumping machinery of complex I. In *Bacillus subtilis* deletion of *mrpA* or *mrpD* from the chromosome resulted in a Na^+ and pH sensitive growth phenotype [1]. In this work the antiporter-like complex I subunits were expressed and their functions were compared in vivo using *B. subtilis* as model system. Expression of MrpA in a *B. subtilis* Δ*mrpD* strain and *vice versa* did not result in any growth improvement under any condition tested. The expressed NuoL could rescue Δ*mrpA* to wild-type growth properties at pH 7.4, but enhanced the growth of Δ*mrpD* only to a lesser extent at this pH. The expressed NuoN could fully restore the wild type properties of Δ*mrpD* in the pH range from pH 6.5 to 7.5. In the Δ*mrpA* strain, expression of NuoN did not improve growth at pH 7.5 but resulted in some growth improvement at pH 6.5. Cells expressing NuoM did not reach wild type growth levels in either deletion strain, but showed some growth improvement under some of the tested conditions. At pH 8.5 no strain could be rescued by any complex I subunit. Taken together, this demonstrates that (i) the antiporter-like Nuo proteins can functionally replace real antiporters and (ii) each of the three complex I antiporter-like subunits has unique functional specializations and operates at different pH. Such pH dependent regulation has previously been described for the Pha antiporter [3], further corroborating that NuoL M and N retain much of their primordial functional mechanism. The implications for the complex I functional mechanism are discussed.

References

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14P.10 Structure and function of *Aquifex aeolicus* sulfide: Quinone oxidoreductase

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Sulfide:quinone oxidoreductases (SQRs) are ubiquitous membrane-bound flavoprotein disulfide reductases (FDRs) that transfer electrons to the membrane quinone pool, thus being essential for sulfide-dependent respiration and photosynthesis. They are also involved in sulfide detoxification, heavy metal tolerance (in yeast) and possibly sulfide signalling (in higher eukaryotes, including humans) [1]. We determined the first complete structure of an SQR at 2.0 Å resolution. We isolated the protein from the native membranes of *Aquifex aeolicus* and could crystallize it in three forms, "as-purified", bound to the substrate quinone and bound to the inhibitor aurachin C [2]. *A. aeolicus* SQR is trimeric and binds the lipid bilayer as an integral monotopic membrane protein, an optimal topology for catalysing the reaction between a soluble and a hydrophobic substrate. The quinone-binding site is located in the membrane-binding domain on the *si*-side of FAD. The quinone ring interacts with conserved F385 and I346 and is protonated upon reduction via G318, K382 and/or neighboring solvent molecules. Sulfide polymerization occurs on the *re*-side of FAD, where the invariant C156 and C347 surprisingly bind the product of the reaction, a polysulfur chain possibly forming an S_8 ring in its mature form. Finally, the structure shows that FAD is covalently connected to the protein in an unprecedented way, via a putative disulfide bridge with C124. Based on our structural observations, we concluded that the SQR reaction needs to be significantly different from that of FDRs and we proposed two alternative reaction schemes. Unexpectedly, there is functional divergence also among SQRs themselves, since the structure of *Acidianus ambivalens* SQR, which also became available recently [3], shows significant difference in the active site in respect to *A. aeolicus* SQR. The structural comparison led us to define new structure-based sequence fingerprints for SQRs and to put the basis for further studies [4]. In particular, the characterization of eukaryotic SQRs is of high priority, because these proteins probably regulate the homeostasis of sulfide, a mediator of sympathetic neurotransmission and a key metabolite in neurodegenerative diseases [5].

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14P.11 Energy conservation by *Rhodothermus marinus* respiratory complex I

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Complex I is the largest and the least understood respiratory chain complex being the electron transfer from NADH to quinone couple to the charge translocation across the membrane. The mechanism of quinone reduction and its coupling to charge translocation is not known. *Rhodothermus marinus* complex I, our model system, is a NADH: menaquinone oxidoreductase and has been extensively characterized. We have made an exhaustive study in order to identify all the subunits present in the complex. We have addressed the charge translocation of complex I using inside-out *R. marinus* membrane vesicles and we observed a NADH-driven sodium ion efflux, together with a proton influx and an inside-positive $\Delta\Psi$. The sodium ion extrusion from the membrane vesicles was due to the activity of complex I, since it was sensitive to its inhibitor rotenone, and it was still observed when the complex I segment of the respiratory chain was isolated by the simultaneous presence of cyanide and external quinones. Using the same approach revealed that H^+ is the electrogenic ion in *R. marinus* complex I. Our results thus show that complex I translocates sodium ions to the direction opposite to that of the establishment of $\Delta\Psi$ by proton translocation, what constitutes the first description of such a process. Moreover, studying the sodium influences of the NADH