ia leading to the proposal that other enzymes involved in oxidative phosphorylation may also be essential (e.g. electron transport chain components). Tibotec BVBA, Johnson and Johnson Phamaceutical Research and Development described the first mycobacterial F_1F_O-ATP synthase inhibitor (TMC207) and showed that TMC207 has fast *in vivo* activity in animal models and TB patients. In this seminar I will discuss new energetic targets and their importance in the adaptation of mycobacteria to persistence i.e. low energy conditions and hypoxia.

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Posters

14P.1 Electrogenicity of succinate: Menaquinone oxidoreductase from *Bacillus subtilis* depends on the direction of electron transfer

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Respiration of *Bacilli* with succinate is known to require energization of the membrane. In order to test whether $\Delta\mu H^+$ can serve as a driving force for the reduction of membrane-bound menaquinone (MQ) by succinate, we have, firstly, investigated a *B. subtilis* strain with cytochrome *bd* as the only terminal oxidase which generates $\Delta\mu H^+$ with low efficiency (1 H^+/e^-). If succinate: menaquinone oxidoreductase (SQR) needs to spend 1 H^+/e^- to reduce MQ, the overall succinate oxidase activity will be electroneutral and conserving no energy. In the minimal growth medium with succinate as a sole energy source, the mutant cells were unable to divide and the subcellular membrane vesicles did not oxidize succinate. These observations are consistent with the energy-consuming mode of the succinate:menaquinone oxidoreductase operation. Second, tightly coupled inside-out membrane vesicles were isolated from *B. subtilis* strain overproducing SQR in which respiration with succinate approached the rate observed with the whole cells. The respiration is coupled to $\Delta\mu H^+$ generation and is completely inhibited by the uncouplers. This finding supports energy-dependence of SQR activity which could be explained either by $\Delta\mu H^+$ consumption in the SQR reaction or by $\Delta\mu H^+$-dependent enzyme conversion to the active form. Third, in the same vesicles, the menaquinol:fumarate reductase (QFR) reaction appears to be electroneutral. Anaerobic reduction of fumarate by NADH proceeds linearly for at least 2 h. The reaction is neither inhibited nor stimulated by the uncouplers, and is not coupled to acidification inside the vesicles. As the fumarate reduction is not inhibited by the uncouplers, conversion of SQR to the active state by $\Delta\mu H^+$ is unlikely. Presumably, SQR in *B. subtilis* has two modes of operation: the reaction in succinate → MQ direction is associated with $\Delta\mu H^+$ consumption, whereas the reverse reaction is electroneutral. Such a behavior resembles the situation with the liposome-reconstituted QFR from *Wolinella succinogenes*, despite the opposite direction of the physiological activity of the two enzymes.

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14P.2 The sodium translocation pathway in Na^+-NQR from *Vibrio cholerae*

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The physiological role of the Na^+-pumping NADH:quinone oxidoreductase (Na^+-NQR) is to convert energy from one form to another. The enzyme uses the energy of a redox reaction, oxidation of NADH and reduction of quinone, to drive the transport of sodium from the inside to the outside of the cell, against its concentration gradient. Turnover of Na^+-NQR produces both, a transmembrane Na^+-gradient and an electrical potential ($\Delta\Psi$), negative inside the cell. Here we report, the characterization of the sodium pathway in Na^+-NQR using a combination of site-directed mutagenesis and kinetics. We characterized several conserved acid residues located in the transmembrane helices of Na^+-NQR that appear to be involved in sodium uptake and release. Seventeen such residues were found, all of them in subunits B, D and E. We then constructed mutants in which these residues were individually replaced by aliphatic groups (alanine or leucine), which should interact poorly with Na^+. Many of these mutations resulted in slowing of the redox reaction (70% to 4% residual quinone reductase activity) and a corresponding decrease in Na^+ translocation. Our results suggest the participation of seven of these residues in the translocation process of sodium. Mutations at NqrB-D397, NqrD-D133 and NqrE-E95 produced a decrease of approximately ten times or more in the apparent affinity of the enzyme for sodium (Kmapp), during turnover, which suggests that these residues may form part of a sodium-binding site. Mutation at other residues, including NqrB-E28, NqrB-E144, NqrB-E346 and NqrD-D88 had a large effect on the quinone reductase activity of the enzyme and its sodium sensitivity, but less effect on the apparent sodium affinity, consistent with a possible role in sodium conductance pathways. Taken together these results, we propose a possible binding site and pathway for sodium in Na^+-NQR.

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14P.3 Heterodimeric bc_1 complex from *Paracoccus denitrificans*: A validation of the half-of-the-sites mechanism

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The bc_1 complex from *Paracoccus denitrificans* is the product of the *fbc* operon, coding for ISP, cytochrome *b* and cytochrome c_1 respectively. In the latter subunit, a very particular feature was identified, an acidic domain showing high homology to the hinge protein in the bovine enzyme or the QCR6p in yeast [1–3]. The domain has been deleted and the resulting complex used to test the half-of-the-sites mechanism [4–7] by constructing a heterodimeric bc_1 in which one of the two monomers carries an inactivating mutation at the Q_O site [8] and therefore, only half of the complex is able to oxidize quinol. The use of the deletion mutant was necessary since the wild type complex resulted to be a stable association of two