oxidation and of proton and sodium transport activities allowed us to propose a model for the mechanism of complex I in which two different ion translocation sites are coupled to electron transfer. Studies performed in the presence of inhibitors corroborate the proposed model. Furthermore, the results obtained for other bacterial complex I open new perspectives on the versatility of this respiratory complex.

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14P.12 The alternative complex III: A different architecture using known building modules

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Until recently cytochrome bc_1 complexes were the only known enzymes able to transfer electrons from reduced quinones to cytochrome *c*. However, a complex with the same activity and with a unique subunit composition was purified from the thermohalophilic bacterium *Rhodothermus marinus* membranes and biochemically, spectroscopically and genetically characterized. This complex was named alternative complex III (ACIII). Later, it was observed that the presence of ACIII is not exclusive of *R. marinus* being the genes coding for this novel complex widespread in the Bacteria Domain. Furthermore, ACIII has been shown to be related to the complex iron–sulfur molybdoenzyme (CISM) family. In this work, the relation of ACIII with members of this family was further investigated by analyzing all the available completely sequenced genomes and a comprehensive description of the state of the art of ACIII is presented. In summary, it was observed that ACIII is a different complex but composed by already known modules, and is thus another example of how nature uses the same structural modules in different contexts according to the metabolic needs.

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14P.13 Fine-tuned cooperative redox networks of multiheme periplasmic cytochromes in *Geobacter sulfurreducens*: Optimal bioenergetic adaptation to environmental changes

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A family of five periplasmic triheme cytochromes (PpcA-E) was identified in the bacterium *Geobacter sulfurreducens* (*Gs*), where they play a crucial role by driving electron transfer from cytoplasm to cell exterior, and assisting the reduction of extracellular acceptors [1]. This work reports the thermodynamic characterization of PpcA, PpcB, PpcD and PpcE using NMR and visible spectroscopies. The heme reduction potentials of these proteins are strongly modulated by heme–heme redox and redox-Bohr (heme-protonated groups) interactions, establishing specific cooperative networks. These networks can be further modulated by the periplasmic pH towards an optimal cellular bioenergetic response to environmental changes. The different functional mechanisms involved suggest that they interact with particular physiological redox partners in the cell. PpcA and PpcD appear to be optimized to interact with redox partners involving e^-/H^+ transfer though via distinct mechanisms. Although no evidence of preferential electron transfer pathway or e^-/H^+ coupling was found for PpcB and PpcE, their working potential ranges suggest that they might also have specific redox partners. The mechanistic properties described for the four *Gs* triheme cytochromes correlate with proteomics and knock-out mutant studies on *Gs* [2,3]. This work constitutes the first step in unraveling the organization of the complex network of redox proteins found in the periplasmic space of the bacterium *G. sulfurreducens*. This functional diversity provides an excellent example as to how structurally related proteins from the same microorganism can interact with particular physiological partners, establishing a rationalization for the co-existence of five homologous periplasmic triheme cytochromes in *Gs*.

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14P.14 Characterization of the supramolecular structure of *Bacillus subtilis* aerobic respiratory chain

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Bacillus subtilis, a Gram-positive soil bacterium, possesses a branched respiratory chain, and is capable of using oxygen or nitrate as terminal electron acceptor. When grown in aerobic conditions, its respiratory chain comprises a type-II NADH: and a succinate:menaquinone oxidoreductase, a bc_1-like menaquinol: cytochrome *c* oxidoreductase and three, possibly four terminal oxygen reductases: a caa_3 cytochrome *c*:oxygen oxidoreductase, and an aa_3 and one or two *bd*-type menaquinol:oxygen reductases [1]. Supramolecular associations between complexes of the electron transfer chain have been demonstrated both in eukaryotes and prokaryotes, enhancing the electron transfer efficiency, and in some cases promoting stabilisation of complex I [2]. Several years ago, a supercomplex composed of a quinol:cytochrome *c* reductase and a cytochrome *c* oxidase was identified in the thermophilic *Bacillus* PS3, showing for the first time the presence of supramolecular associations of respiratory chain complexes in the *Bacillus* genus [3]. We have carried out the aerobic growth of *B. subtilis* 168, promoted cell disruption by means of a French press and isolated the membranes for further studies. Characterization of the expressed complexes was performed by UV–visible spectrophotometry and substrate:oxygen polarographic measurements of the respiratory chain enzymatic activities using specific inhibitors. To investigate supramolecular associations between these complexes, we have performed BN-PAGE and detected in gel activity of the different respiratory enzymes. Our preliminary results suggest that also the aerobic respiratory chain of the mesophilic *B. subtilis* is organized in supercomplexes.

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14P.15 Supramolecular organization of the aerobic respiratory chain of *Escherichia coli*

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The composition of the *Escherichia coli* aerobic respiratory chain varies according to the oxygen tension of growth and relies essentially on the expression of at least five proteins, namely type I and II NADH:quinone oxidoreductases, succinate:quinone oxidoreductase, cytochrome *bd* oxygen reductase and cytochrome bo_3 oxygen reductase. The arrangement of these enzymes in the cytosolic membrane is still controversial, mostly due to increasing evidence suggesting their organization into supramolecular assemblies, in respiratory chain complexes from other organisms. To investigate if such organization could be detected in the aerobic respiratory chain of this bacterium, membranes of *E. coli* K-12 solubilized with digitonin were analyzed by BN-PAGE followed by *in-gel* activity and heme staining detection. The resulting bands were also loaded into 2D-Tricine-SDS-PAGE and 2D-CN-PAGE, transferred to PVDF membranes and immunodecorated with polyclonal antibodies against type I NADH:quinone oxidoreductase, succinate:quinone oxidoreductase and cytochrome bo_3 oxygen reductase. The expected trimeric and dimeric assemblies of succinate:quinone oxidoreductase and cytochrome bo_3 were detected as well as four bands presenting NADH:NBT oxidoreductase activity. Two of the NADH:NBT oxidoreductase stained bands stained also with antibodies against the type I NADH:quinone oxidoreductase, the succinate:quinone oxidoreductase and the cytochrome bo_3 oxygen reductase, strongly suggesting that the *E. coli* respiratory chain harbours at least a supercomplex containing these three enzymes. The BN-PAGE results are further corroborated by sucrose gradient analysis and gel filtration column purification [1].

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14P.16 Redox processes, energy transfer and molecular hydrogen production studies with *Escherichia coli* at different pH

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Escherichia coli perform sugar or glycerol fermentation upon which redox processes, energy transfer and H_2 production are of interest. Two aspects are under our study: (1) In neutral and slightly alkaline medium *E. coli* carry out H^+–K^+-exchange through the F_0F_1-ATPase and the TrkA system when energy for K^+ uptake is transferred from F_OF_1 by dithiol-disulfide interchange between these complexes; data obtained support this idea. A relationship between H^+–K^+-exchange and H_2 production by formate hydrogen lyase (FHL) might be mediated by redox equivalent supply from formate for a dithiol–disulfide interchange. Moreover, proton-motive force generated by F_0F_1 may be required for transport of formate into cells and for FHL activity. However, in acidic medium, F_0F_1 is likely to be also necessary for H_2 production. (2) Two forms of FHL-1 and FHL-2, which constituted by formate dehydrogenase H encoded by the *fdh* operon and hydrogenases 3 (Hyd-3) or 4 (Hyd-4), encoded by the *hyc* and *hyf* operons, respectively, are responsible for H_2 production. Since Hyd-3 and Hyd-4 are encoded by genes of different operons and are characterized by different subunit composition and organization in the membrane it is assumed that these forms are functionally active under different conditions and therefore they play distinct roles in bacteria. Our study with *E. coli* grown under glucose fermentation at neutral and slightly alkaline pH has shown that H_2 production was not observed in *fdhF* and *hyf*-mutants lacking Fdh-H large subunit or Hyd-4 subunits, respectively. Interestingly, production of H_2 was detected in various *hyc* mutants lacking Hyd-3 subunits, but it was not formed in the *hycB* mutant. These results suggest that H_2 production by *E. coli* involves FHL-2; HycB requirement subunit suggests that it represents a constituent of Fdh-H. The physiological role of FHL-2 may be required for generation of CO_2 to use in the formation of oxaloacetate from phosphoenolpyruvate during fermentation. At acidic pH H^+–K^+-exchange by *E. coli* was distinguished: K^+ uptake by the Kup system was markedly lower in *hyfR* and *hyfB-R* but not in *hycE* or *hyf A-B* mutants and H_2 production was significantly suppressed in the *hyc* but not *hyf* mutant. The *hyfB-R* genes are suggested to be expressed under low pH. These genes or their gene products are possible to interact with the gene coding for Kup or directly with Kup.

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14P.17 The type II NADH: Quinone oxidoreductase of *Mycobacterium tuberculosis*: A novel drug target for an age-old problem

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The *Mycobacterium tuberculosis* (Mtb) respiratory chain presents unique antitubercular drug development opportunities. In addition to a 14 subunit Complex I, the Mtb Electron Transfer Chain contains a single subunit type II NADH:quinone oxidoreductase (Ndh). Transcriptional studies, animal infection models and biochemical analyses have shown this metabolic choke point to be essential [1,2]. It is a major contributor to the viability-sustaining membrane potential ($\Delta\Psi m$) regardless of metabolic state and consequently inhibition induces death in actively-growing and dormant Mtb. Targeting Ndh will mitigate failings of current therapies, circumventing current resistance mechanisms and sterilising dormant populations. Heterologously expressed Mtb Ndh has been successfully isolated from the double NADH-dehydrogenase knockout *E. coli* strain ANN0222 (*nuoB*::*nptl-sacRB*, *ndh*::*tet*, supplied by Prof. T. Friedrich, Freiburg) and is catalytically active. This activity is rotenone and piercidin A insensitive, however, in line with recent literature [1,2] Mtb Ndh is sensitive to the phenothiazines trifluoperazine and thioridazine. To probe the Ndh quinone binding site a conserved tryptophan (W276) postulated to be critical to enzyme–substrate interaction was mutated to Ala, Leu, and Phe. W276A and W276L mutants are inactive whilst W276F displays minimal activity suggesting that *pi-pi* stacking interactions between W276and substrate aromatic rings are critical to catalysis. A direct positive correlation between enzyme inhibition and Mtb bactericidal activity has been