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.