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Review

Multiple Rieske/cyt*b* complexes in a single organism ☆

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ABSTRACT

Most organisms contain a single Rieske/cyt*b* complex. This enzyme can be integrated in any respiratory or photosynthetic electron transfer chain that is quinone-based and sufficiently energy rich to allow for the turnover of three enzymes — a quinol reductase, a Rieske/cyt*b* complex and a terminal oxidase. Despite this universal usability of the enzyme a variety of phylogenetically distant organisms have multiple copies thereof and no reason for this redundancy is obvious. In this review we present an overview of the distribution of multiple copies among species and describe their properties from the scarce experimental results, analysis of their amino acid sequences and genomic context. We discuss the predicted redox properties of the Rieske cluster in relation to the nature of the pool quinone. It appears that acidophilic iron-oxidizing bacteria specialized one of their two copies for reverse electron transfer, archaeal Thermoprotei adapted their three copies to the interaction with different oxidases and several, phylogenetically unrelated species imported a second complex with a putative heme c_i that may confer some yet to be determined properties to the complex. These hypothesis and all the more the so far completely unexplained cases call for further studies and we put forward a number of suggestions for future research that we hope to be stimulating for the field. This article is part of a Special Issue entitled: Respiratory complex III and related bc complexes.

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1. Introduction

Rieske/cyt*b* complexes are present in most organisms that rely on chemiosmosis and produce their ATP via a transmembrane proton gradient that drives an H^+-ATPase. Rieske/cyt*b* complexes have (so far) been found in at least some species of all phyla to the exception of methanogens, Archaeoglobi, Thermococci, Nanoarchaeota, Fibrobacteria, Elusiomicrobia, Dictyloglomi, Fusobacteria, Synergistetes, Tenericutes, Thermotogae and Enterobacteria.

Although phylogeny cannot conclusively answer questions related to enzyme function, it is a valuable tool to put available results in a broader context, to detect open questions and to search for organisms that may be well-suited for future functional studies. With this article we intend to emphasize the complementarity between phylogeny and functional studies.

Phylogenetic analyses of cytochrome *b* from Rieske/cyt*b* complexes over the past 15 years consistently produced phylogenetic trees that correspond to available 16S-rRNA trees with respect to the cleavage between Archaea and Bacteria and the branching order of many bacterial phyla: Actinobacteria and *Thermus*/*Deinococcus* are found on low branches, the branching order among proteobacteria is conserved and Heliobacteria, Bacilli and cyanobacteria group together on both trees. The core of the complex i.e. the transmembranous cytochrome *b* and the Rieske subunit were therefore proposed to have already been present in the Last Common Universal Ancestor and to have evolved ever since mainly by vertical evolution [1–3]. The consensus of phylogenetic trees notwithstanding, a substantial differing scenario has been put forward recently [4], stipulating a late origin of the enzyme followed by horizontal distribution. The crucial parameter to deduce ancestry of an enzyme is the positioning of the root in its phylogenetic tree. The root cannot be inferred from the tree, unless an outgroup can be included in the phylogenetic analysis. The fact that the Rieske protein also occurs in arsenite oxydases allows the reconstruction of a composite phylogeny of the two enzymes and the mutual rooting of the individual trees [5]. These two enzymes were found to root each other in between Archaea and Bacteria, a topology straightforwardly rationalized by the presence of both enzymes in the Last Common Universal Ancestor. In the framework of this interpretation a few cases of lateral gene transfer can be clearly detected by the divergent position of the species on protein and 16S-rRNA trees with Aquificales [6] and Haloarchaea [1] as prominent examples. Some groups of Bacteria are found on various positions on different 16S-rRNA trees and their position on the tree of cytochrome *b* of Rieske/cyt*b* complexes is supported by low bootstrap values only and is furthermore dependant on the subset of organisms included. This holds for Chlorobiacaea and Chlamydiae. Fig. 1 shows a phylogenetic tree constructed from cytochrome *b* sequences. Species are represented in blue, unless their Rieske/cyt*b* complex originates from lateral gene transfer (yellow). A gray background indicates that the history of the Rieske/cyt*b* complex in these species is not yet clear.

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Rieske/cyt*b* complexes are transmembranous enzymes of respiratory chains that react with quinones and electron acceptor proteins such as cytochromes, cupredoxin-type copper proteins or high potential iron–sulfur proteins. They do not metabolize exogenous substrates and therefore operate necessarily at the interior of bioenergetic electron transfer chains. Enzymes reducing quinones have to be present further up the chain and enzymes reducing a terminal electron acceptor need to follow. The Rieske/cyt*b* complex can be integrated in any respiratory chain that operates with quinones whatever the nature of the initial electron donor and terminal acceptor, provided they are driven by a sufficiently high redox span to allow for the turnover of at least three enzymes [7]. This universal usability of the Rieske/cyt*b* complex justifies the presence of a single copy of this enzyme in organisms that have a sufficiently energy-rich, quinone involving bioenergetic metabolism with a redox-wise homogenous quinone pool.

All Rieske/cyt*b* complexes studied so far function according to the Q-cycle: they release four protons on the outside of the membrane, take up two protons from the inside and transfer two electrons to periplasmic electron acceptors. They thereby contribute to the build-up of a transmembrane proton gradient that drives the ATPase. The number of protons actually useful for ATP production depends on the buffering capacity and the size of the compartments on both sides of the membrane and varies from two to four per two electrons transferred to the Rieske cluster. During turnover of Rieske/cyt*b* complexes two quinols are oxidized in a quinol oxidizing site on the positive side of the membrane and one quinone is reduced in a quinone reducing site on the negative side of the membrane. This enzyme therefore has the particularity to both reduce and oxidize a molecule of the same chemical nature — a quinone from the quinone pool of the organism. The energy to drive this reaction against the membrane potential comes from the bifurcated

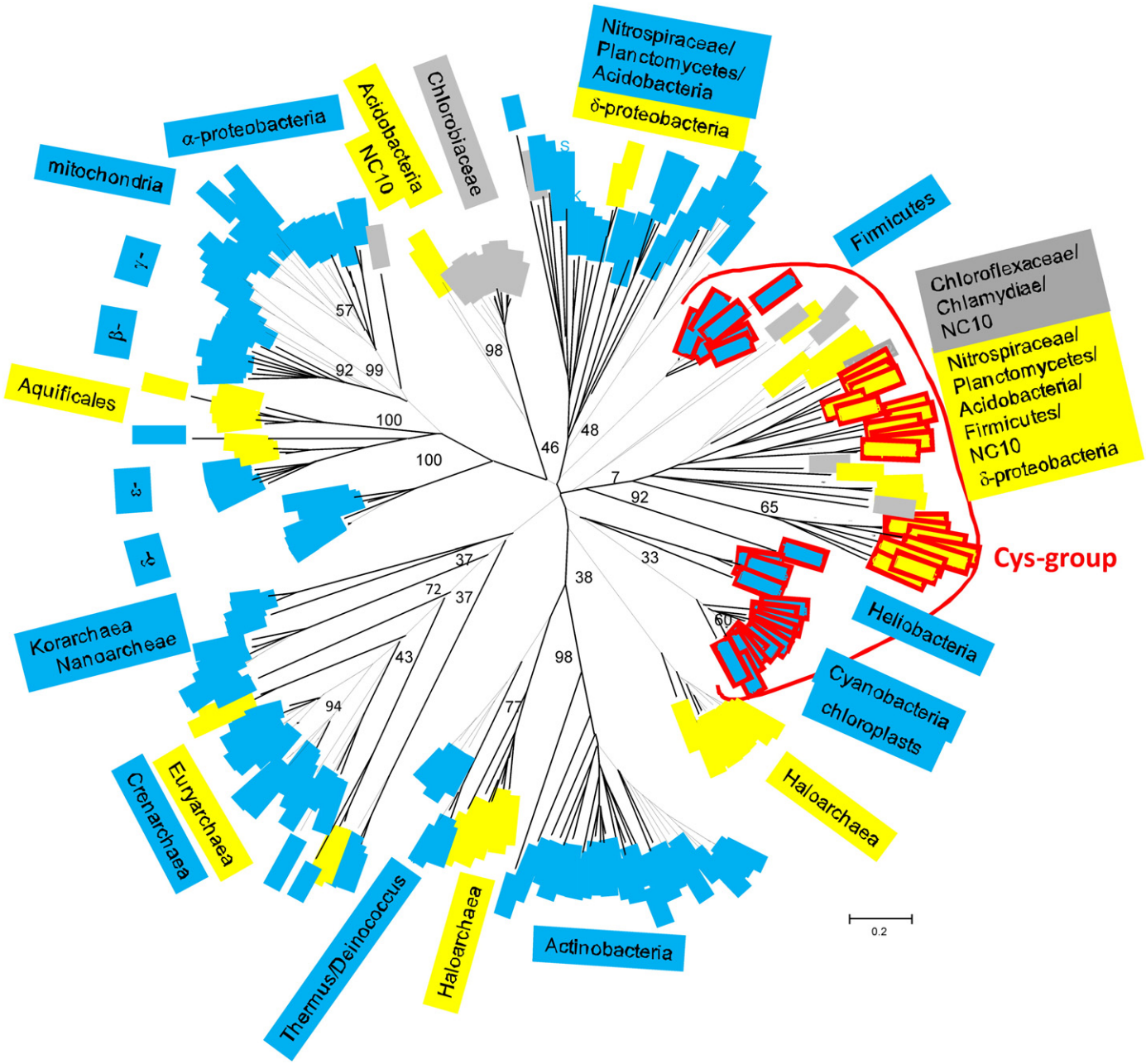


Fig. 1. Phylogenetic tree of the cytochrome *b* subunit of Rieske/cyt*b* complexes. Species that occupy a position on the tree similar to their position on the 16SrRNA tree are shown in blue, and species that acquired a Rieske/cyt*b* complex via lateral gene transfer are shown in yellow. Gray indicates species for which the position on the 16SrRNA tree and the cytochrome *b* tree is not yet resolved. The names of the phyla are indicated. Red boxes mark species that have a conserved cysteine residue in their cytochrome b_6 sequence, homologous to the one that links heme c_i in cyanobacteria and chloroplasts. Sequence alignments and tree building were done as described in [1].

electron transfer of the quinol in the Q_o site that shuttles one electron to the high potential acceptors, i.e. the Rieske protein and its subsequent electron acceptors while taking advantage of the low redox potential of the second electron to reduce the quinone on the other side of the membrane via two hemes placed across the membrane. The high redox potential of the first electron makes the electron transfer to the Rieske protein an uphill reaction that nonetheless occurs readily since the second electron transfer reaction to the Q_i site quinone is exergonic [8]. The fact that the driving force for the quinol oxidation and therefore the turnover of the complex is linked to the departure of the second electron from the Q_o site quinol ensures the observed strict bifurcation of the electron transfer reactions from the Q_o site quinol to its respective acceptors. A fine-tuned redox balance between the redox potential of the quinone to be oxidized and those of its electron acceptors, i.e. the Rieske protein, the *b*-hemes and the acceptor quinone is necessary for this cycle to turn over efficiently. Indeed, for organisms studied so far, a correlation between the redox potential of the quinone pool and the redox potential of the Rieske protein was observed.

The redox potential of the quinone depends on its chemical nature. Menaquinone (MK) is the most widespread quinone type among all organisms and has a redox midpoint potential of −70 mV (at pH 7) [9]. In several phylogenetic groups widely distributed in the extant biosphere high potential quinones are present such as ubiquinone (UQ) in proteobacteria and mitochondria, plastoquinone (PQ) in cyanobacteria and chloroplasts and calderiellaquinone (CQ) e.g. in Sulfolobales, which all have a redox midpoint potential of +100 mV at pH 7 [9]. Furthermore the redox potentials of rhodoquinone (RQ) found in some alpha- and betaproteobacteria, (−65 mV at pH 7 [10,11]), demethylmenaquinone (DMK) present in some Enterobacteria, (+30 mV at pH 7 [12,13]) and in Aquifex, (+9 mV at pH 7 [14]) and methylmenaquinone (so far reported at −220 mV at pH 7 in Wolinella [15]) have been determined. Some quinone species have been detected for which the redox midpoint potentials are still unknown such as thermoplasmaquinone 'TPQ' [16], sulfolobusquinone 'SQ' [17] and methylthionaphthoquinone 'MTK' [18].

A correlation has been established between the nature of two amino acids in close vicinity of the [2Fe2S] cluster of the Rieske protein and its redox midpoint potential [19–22]. In Fig. 2 the organisms that harbor Rieske proteins predicted to have redox potentials above +260 mV are highlighted in orange (key amino acids: serine and tyrosine), potentials around +150 mV are in blue (glycine and phenylalanine) and intermediate potentials are in light orange (serine and phenylalanine) and light blue (glycine or alanine and tyrosine). Phyla reported to have low potential quinones, i.e. MK or DMK, are marked in blue and those reported to have high potential quinones, i.e. UQ, PQ, CQ are marked in red. In most cases the redox potential of the quinone corresponds to the predicted redox potential of the Rieske protein.

The vast majority of prokaryotes are multivalent with regard to redox substrates, a property crucial for survival in fluctuating environments. These redox substrates span various ranges of electrochemical potentials. As mentioned above, presence and functionality of a Rieske/cyt*b* complex is only possible above a minimum threshold of electrochemical potential difference between the donor and the acceptor substrate couple. Such suitable redox spans exist in very different redox regimes and are indeed exploited by life [7]. Some proteobacteria are known to employ quinones with different redox potentials to account for various redox regimes [21,23–25]. Given the above detailed necessity for redox fine-tuning between the Rieske/cyt*b* complex and its electron transfer partners, one might expect the presence of a dedicated Rieske/cyt*b* enzyme for every quinone-type operating in an electron transfer chain. This is not the case and organisms known to harbor both, high- and low potential quinones such as gammaproteobacteria [21] or *Rhodospirillum rubrum* [26] have a single Rieske/cyt*b* complex. In the studied cases the Rieske/cyt*b* complex has been shown to efficiently turn over with one of the quinone species only.

Most studied organisms contain indeed a single Rieske/cyt*b* complex and this seemed to be the rule to the exceptions of *Acidithiobacillus* [27,28] and *Sulfolobus* [29,30], which were treated as outliers in the past. In our two most recent updates on the phylogeny of Rieske/cyt*b* complexes [1,31], however, we were confronted with an increasing number of species coding for two or more Rieske/cyt*b* complexes in their genomes. Most of these cases correspond to species for which only scant or no data on energy metabolism are available. A survey of the literature came up with more species for which the presence in the genome of genes coding for more than one Rieske/cyt*b* complex was mentioned: *Metallosphaera* [32], *Leptospirilla* [33], *Kuenenia* [34], *Scalindula* [35], *Blastopirellula* [31], *Solibacter* [31], *Candidatus Nitrospira defluvii* [36,37], *Candidatus Chloracidobacterium thermophilum* [38], and Haloarchaea [1,39].

In general, the presence of multiple copies of bioenergetic enzymes is attributed to the metabolic versatility of the respective organism, with *Shewanella oneidensis* as a frequently cited example [40,41]. In the light of the above described tight correlation between physicochemical parameters of the Rieske/cyt*b* complexes and the bioenergetic chains they are embedded in, we feel that the phenomenon of multiple Rieske/cyt*b* complexes in selected species deserves a more dedicated inventory. The purpose of this contribution therefore is to detect and summarize such cases, review current knowledge of the very few investigated examples, formulate open questions and define specific research objectives that we hope will be stimulating for research into the significance of redundancy of Rieske/cyt*b* complexes in single organisms.

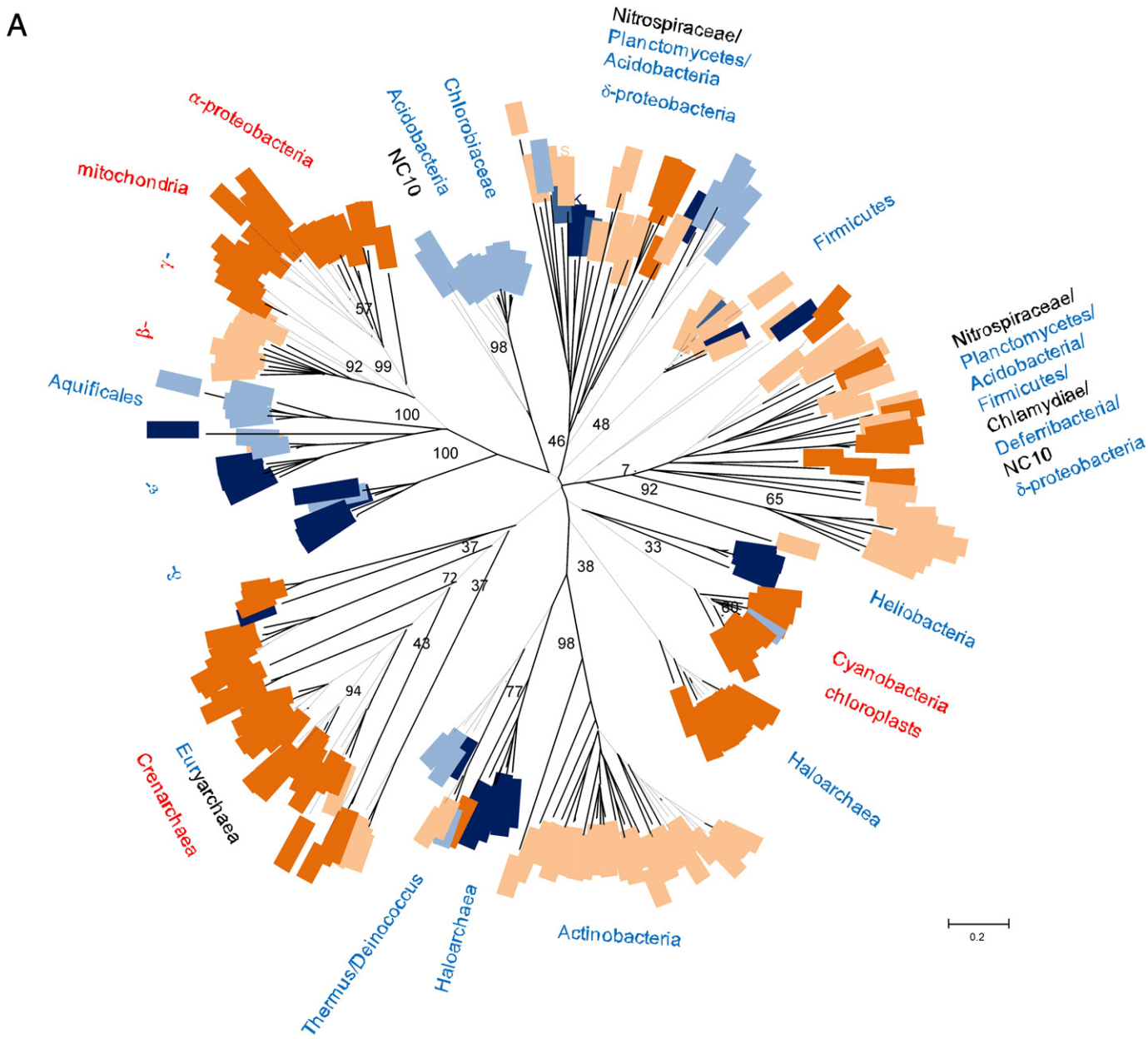
2. Multiple Rieske/cyt*b* complexes are widespread among phyla

We performed a BLAST search on currently completely sequenced genomes of Bacteria and Archaea available as of September 2012 using different Rieske proteins or cytochromes *b* as queries and came up with so far unpublished cases of multiple copies of this enzyme among Acidobacteria, Thermoplasmata, Candidate division NC10, epsilon- and deltaproteobacteria (Table 1).

We restricted our analysis to the presence of multiple copies of Rieske/cyt*b* complexes which implies that we will not discuss the case of multiple Rieske proteins (as seen in Cyanobacteria [42] and alpha- and betaproteobacteria [43]) nor that of multiple cytochromes *b* (as present in the Actinobacteria *Kribella flavida*, *Streptosprangium roseum*, *Thermomonospora curvata* and in *Gloeobacter violaceus* [44]) nor copies of enzymes that are missing the equivalent of SUIV (as detected in *Singulisphaera acidiphila*, *Pirellula staleyi*, planctomycete KSU-1, *Desulfomonile tiedjei*, *Geobacter sulfurreducens*, and *Geobacter metallireducens*) nor truncated copies of the enzyme as found in the genome of *Hydrogenivirga* sp. 128-5-R1-1.

With the exception of *Acidithiobacillus* [27,45,46] and *Sulfolobus* [47–57], none of the organisms harboring more than one copy of Rieske/cyt*b* complexes has been investigated by biochemical or biophysical studies. Our present investigation therefore relies on sequence analysis of the subunits of the complexes, their genomic context and the sparsely available microbiological, transcriptomic and proteomic studies. Some characteristics of Rieske/cyt*b* complexes can easily be deduced from the sequences. Cytochrome *b* is split into two proteins in some organisms. One protein contains the first four transmembrane helices

Fig. 2. A: Phylogenetic tree of the cytochrome *b* subunit of Rieske/cyt*b* complexes. Species that harbor a Rieske subunit with a sequence signature indicating a high redox potential of the Rieske cluster (SY) are marked in orange, low potential in blue (GF/AF) and intermediate cases are marked in light orange (SF) and light blue (GY/AY). The name of the phylum is written in red if the pool quinone of the species is ubiquinone, plastoquinone or calderiellaquinone, in blue if it is menaquinone or DMK and in black if it is still to be determined. B: Schematic representation of the redox range covered by Rieske proteins with different sequence signatures and redox potentials of the quinones. The arrows visualize the redox potential difference available between the quinone and the Rieske cluster that drives the Q-cycle.


A
Nitrospiraceae/
Planctomycetes/
Acidobacteria
δ-proteobacteria
Chlorobiaceae
Acidobacteria
NC10
α-proteobacteria
mitochondria
γ-
β-
Aquificales
ε-
δ-
Firmicutes
Nitrospiraceae/
Planctomycetes/
Acidobacteria/
Firmicutes/
Chlamydiae/
Deferribacteria/
NC10
δ-proteobacteria
Heliobacteria
Cyanobacteria
chloroplasts
Haloarchaea
Actinobacteria
Haloarchaea
Thermus/Deinococcus
Euryarchaea
Crenarchaea
0.2

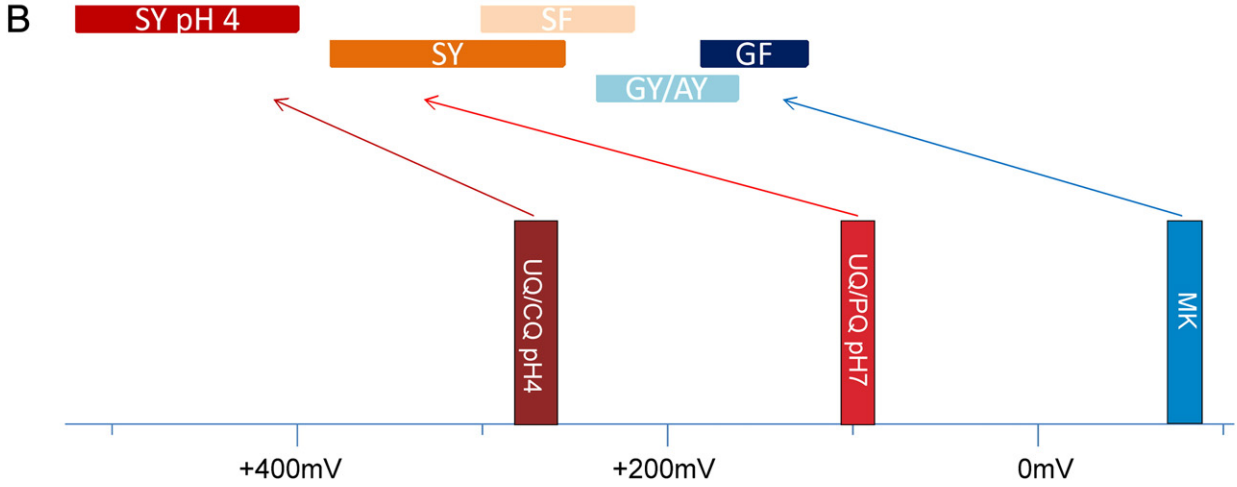

B
SY pH 4
SF
SY
GF
GY/AY
UQ/CQ pH4
UQ/PQ pH7
MK
+400mV
+200mV
0mV

Table 1
Sequence characteristics of Rieske/cyt*b* complexes discussed in this paper. In the last column the quinone type present in the species is indicated, when known.

Species	Phylum	Sequence signature of the Rieske protein	Nature of cytochrome *b*	Presence of Cys	Quinone type
C. *Kuenenia stuttgartiensis*	Planctomycetes	SF	b_6/SUIV	Cys	MK[a]
		SY	b_6/SUIV	Cys	
		GF	*b*		
Planctomycete KSU-1	Planctomycetes	SF	b_6/SUIV	Cys	
		SY	b_6/SUIV	Cys	
		GF	*b*		
Blastopirellula marina	Planctomycetes	SF	b_6/SUIV	Cys	MK [57]
		SF	*b*		
		SF	*b*		
C. *Methylomirabilis oxyfera*	Candidatus phylum NC10	SF	b_6/SUIV	Cys	
		SF	*b*		
		GY	*b*		
Terriglobus saanensis	Acidobacteria	GF	*b*		
		GY	*b*		
C. *Chloracidobacterium thermophilum*	Acidobacteria	SF	*b*		MK [83–85]
		GY	*b*		
Solibacter usitatus	Acidobacteria	SY	b_6/SUIV		
		SF	*b*		
		SF	*b*		
		GY	*b*		
Desulfomonile tiedjei	Deltaproteobacteria	GF	*b*		
		SF	*b*		
Geobacter daltonii	Deltaproteobacteria	SF	b_6/SUIV	Cys	
		SF	b_6/SUIV		
Geobacter sp M21	Deltaproteobacteria	SF	b_6/SUIV	Cys	
		GF	*b*		
Geobacter sp M18	Deltaproteobacteria	SF	b_6/SUIV	Cys	
		GF	*b*		
Geobacter bemidjiensis	Deltaproteobacteria	SF	b_6/SUIV	Cys	MK [89]
		GF	*b*		
Geobacter uranireducens	Deltaproteobacteria	SF	b_6/SUIV	Cys	
		GF	*b*		
Geobacter metallireducens	Deltaproteobacteria	SF	b_6/SUIV		MK [89]
		GY	*b*		
Sulfurospirillum deleyianum	Epsilonproteobacteria	GF	*b*		
		GF	*b*		
Acidithiobacillus ferrooxidans	Gammaproteobacteria	SY	*b*		
		SY	*b*		
Acidithiobacillus ferrivorrans	Gammaproteobacteria	SY	*b*		
		SY	*b*		
Leptospirillum ferrooxidans	Nitrospiraceae	SY	b_6/SUIV	Cys	
		SY	*b*		
Leptospirillum ferrodiazotrophum	Nitrospiraceae	SY	b_6/SUIV	Cys	
		SY	*b*		
Leptospirillum rubarum	Nitrospiraceae	SY	b_6/SUIV	Cys	
		SY	*b*		
C. *Nitrospira defluvii*	Nitrospiraceae	SY	*b*		
		SF	*b*		
Halorhabdus utahensis	Haloarchaea	SY	b_6/SUIV		
		GF	*b*		
Haloarcula marismortui	Haloarchaea	SY	b_6/SUIV		
		GF	*b*		
Halomicrobium mukohataei	Haloarchaea	SY	b_6/SUIV		
		GF	*b*		
Halogeometricum borinquense	Haloarchaea	SY	b_6/SUIV		
		GF	*b*		
Halorubrum lacusprofundi	Haloarchaea	SY	b_6/SUIV		
		GF	*b*		
Haloferax volcanii	Haloarchaea	SY	b_6/SUIV		MK [19]
		SY	*b*		
		GF	*b*		
Sulfolobus acidocaldarius	Thermoprotei	SY	*b*		
		SY	*b*		
		SY	*b*		
Sulfolobus solfataricus	Thermoprotei	SY	*b*		SQ, CQ [26]
		SY	*b*		
		SY	*b*		
Sulfolobus islandicus	Thermoprotei	SY	*b*		
		SY	*b*		
		SY	*b*		
Sulfolobus tokodaii	Thermoprotei	SY	*b*		
		SY	*b*		
		SY	*b*		
Metallosphaera sedula	Thermoprotei	SY	*b*		
		SY	*b*		
		SY	*b*		

Table 1 (*continued*)

Species	Phylum	Sequence signature of the Rieske protein	Nature of cytochrome *b*	Presence of Cys	Quinone type
Metallosphaera cuprina	Thermoprotei	SY	*b*		
		SY	*b*		
		SY	*b*		
Caldivirga maquilingensis	Thermoprotei	SY	*b*		
		SF	*b*		
Thermoproteus uzoniensis	Thermoprotei	SY	*b*		
		SF	*b*		
Thermoplasma acidophilum	Thermoplasmata	SY	*b*		MK, TPK, MTK [12]
		SY	*b*		
Thermoplasma volcanicum	Thermoplasmata	SY	*b*		
		SY	*b*		

[a] Jan T. Keltjens, personal communication.

and the His residues that are axial ligands to the two heme cofactors and is named cytochrome b_6. The second one, called subunit IV, contains the remaining transmembrane (mostly three) helices and does not bind any cofactors. Many of these split cytochromes *b* feature a conserved cysteine residue close to the N-terminus of cytochrome b_6. In Cyanobacteria and chloroplasts the crystal structure showed [58,59] that this cysteine covalently binds a heme (heme c_i) that is situated in the quinone reducing site. The presence of this heme was confirmed by biochemical and biophysical experiments in Bacilli [60] and Heliobacteria [61]. Its function is still enigmatic. Organisms that have Rieske/cyt*b* complexes with the conserved cysteine residue are boxed in red on the tree in Fig. 1.

The same tree with a different color code is represented in Fig. 3, highlighting organisms with several copies of Rieske/cyt*b* complexes. For some species both copies are phylogenetically close, indicating that they originate from gene duplications in an ancestor of the species or the phylum. This seems to be the case for *Acidithiobacillus*, *Terriglobus*, *Nitrospira*, *Sulfurospirillum* and Thermoprotei, shown in different shades of blue. Other organisms have two copies of the complex localized in different regions of the tree. If one of the copies is situated in a position corresponding to the 16SrRNA position we suppose it to be vertically inherited and the other one is interpreted as resulting from an event of lateral gene transfer. This is the case in *Geobacter*-species, depicted in violet and in *Leptospirillum*, *Kuenenia*, KSU-1, *Solibacter* and *Blastopirellula*, shown in different shades of green. The three copies of the enzyme present in *Methylomirabilis* (black) are located in different regions of the tree. Thermoplasmata (brown) and Haloarchaea (orange) have both copies of the enzyme situated in positions on the tree that do not correspond to the 16S-rRNA positions of the organisms and we therefore suspect both copies to have arisen from lateral gene transfer events. Whereas Thermoplasmata got both copies from Archaea, Haloarchaea imported them from Bacteria. The vast majority of imported second copies of Rieske/cyt*b* complex are situated in a single group on the phylogenetic tree of cytochrome *b* among the organisms that have a split cytochrome *b* and a conserved cysteine residue potentially ligating a heme c_i. Throughout this article we will refer to this group as the 'Cys-group' (see Fig. 1).

Whether the enzyme was acquired via lateral gene transfer or vertically inherited by an organism, the presence of multiple copies suggests that a specialization of the individual enzymes took place. During our analysis we came up with four possible reasons for a specialization of individual copies: optimization of two copies for forward and reverse electron transfer, respectively; adaptation of several copies to the interaction with a specific terminal electron acceptor in a supercomplex; a yet to be determined property conferred to one of the copies of the enzyme present in an organisms by the presence of heme c_i; an adaptation of the complex to the redox state of the quinone pool by a variation in the redox midpoint potential of the Rieske iron sulfur cluster. Support for these hypotheses as well as so far unexplained cases will be presented in the following.

Writing this article we were confronted with the problem of how to organize information available from the rather scarce experimental results, analysis of amino acid sequences and the genomic context of the respective genes. We finally decided to go for an ordering based on phylogenetic criteria. This may be tedious to read for a lectureship interested in the Rieske/cyt*b* complexes in general but should facilitate accessing the relevant data for colleagues working on selected organisms.

Table 1 summarizes the species names of organisms harboring multiple copies of the Rieske/cyt*b* complex, the sequence signature of their Rieske protein, the nature of their cytochrome *b* (split or unsplit), the presence of the conserved cysteine residues as a putative ligand to heme c_i and, if available, the quinone type detected in the organisms. Fig. 4 visualizes the genomic context of Rieske/cyt*b* coding genes. Supplementary Fig. S1 shows the phylogenetic tree with all the species names indicated and Fig. S2 shows the underlying sequence alignment.

2.1. Planctomycetes

Blastopirellula marina has one Rieske/cyt*b* gene cluster with a split cytochrome *b* and a cysteine that indicates the possible presence of a heme c_i and two more genes coding for cytochrome *b* and Rieske proteins which are not clustered. Rieske proteins and cytochromes *b* are generally encoded side by side in the genomes of Prokaryotes (see Fig. 4). The rare exceptions are found among Haloarchaea, Thermoprotei (see below) and a proteobacterium [21] where biochemical or phylogenetic information indicates their assembly into a Rieske/cyt*b* complex. For *B. marina* we have no hint as to the assembly of the subunits encoded by the second and third Rieske proteins and cytochromes *b*. The organism contains MK [62] as do all Planctomycetes investigated so far [62,63]. Genes for an ACIII are also found in its genome. ACIII is a membrane-bound multi subunit enzyme proposed to fulfill the same roles as Rieske/cyt*b* complexes, that is quinol oxidation, reduction of a soluble periplasmic electron carrier and possibly transmembrane proton transfer [64].

Anammoxbacteria are slowly growing organisms that were reported to be responsible for 50% of marine N_2 production [65]. They are the only other Planctomycetes known to have more than one Rieske/cyt*b* complex. Three copies are found in the genomes of *Kuenenia stuttgartiensis* and KSU-1 and *Scalindula* was reported to have genes for several Rieske/cyt*b* complexes [35] but the genome is not yet accessible. MK is the only quinone that has been found in several Planctomycetes (*Gemmata aurantiaca* [63], *B. marina* [62], *Planctomyces brasiliensis* [62] and in *K. stuttgartiensis* (Jan T. Keltjens, personal communication)).

All Planctomycetes have an outer protein cell wall, a cytoplasmic membrane, a paryphoplasm, an intracytoplasmic membrane and a riboplasm [41]. In Anammoxbacteria the anammoxosome, an organelle with a contiguous membrane that takes up 30–70% of the cell volume is localized in the riboplasm. Ladderane lipids are present in Anammoxbacteria but not in other Planctomycetes and make up the majority of lipids of the anammoxosome membrane. They are more rigid than other lipids and are reported to render membranes less permeable to diffusion of ions [66]. Several observations indicate that the energy metabolism takes place in the anammoxosome. Cytochrome *c* makes up 10%–30% of the total protein mass of

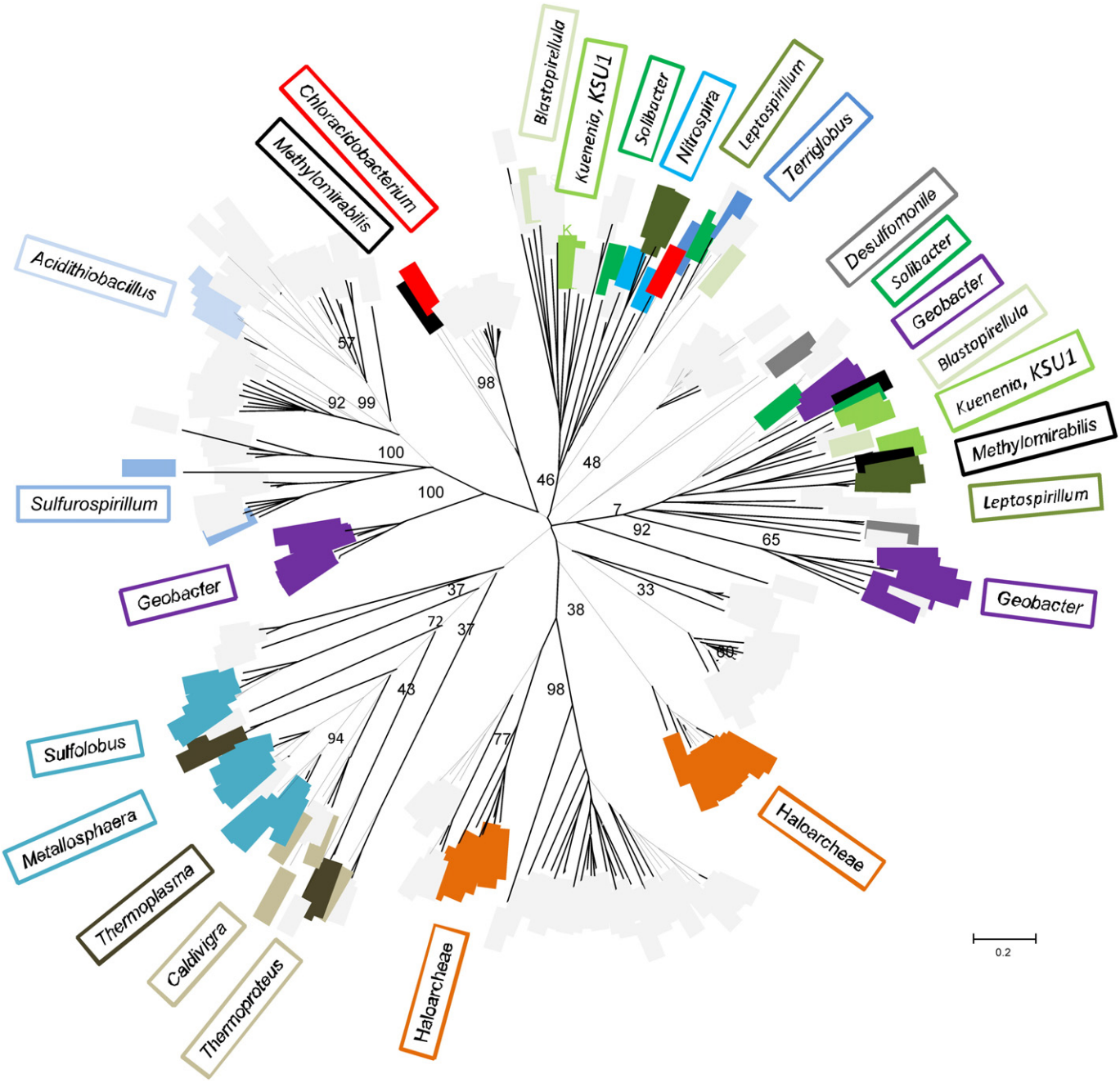


Fig. 3. Phylogenetic tree of the cytochrome *b* subunit of Rieske/cyt*b* complexes. Occurrences of multiple copies of Rieske/cyt*b* complexes in a single organism are highlighted by colors. Different shades of blue stand for *Acidithiobacillus*, *Terriglobus*, *Nitrospira*, *Sulfurospirillum*, *Metalosphaera* and *Sulfolobus*, shades of green for *Leptospirillum*, *Kuenenia*, KSU-1, *Solibacter* and *Blastopirellula*, violet for Geobacter species, brown for *Thermoplasma*, light brown for *Caldivigra* and *Thermoproteus*, orange for Haloarcheae, black for *Methylomirabilis*, red for *Chloracidobacterium* and dark-gray for *Desulfomonile*.

Anammoxbacteria. It is exclusively found in the anammoxosome, mainly close to the inner side of the membrane [67]. A multiheme (2, 6 or 8 hemes) *c*-type cytochrome is part of the genomic context of all Rieske/cyt*b* complexes of *K. stuttgartiensis*. In one of the three complexes a diheme cytochrome *c* is fused to the cytochrome *b* subunit. Consequently, the Rieske/cyt*b* complex should be in the anammoxosome membrane with the globular domain of the Rieske protein facing inside, in agreement with the Tat-signal sequence present in all Rieske subunits that targets the subunit for transfer over a membrane. pH values of 6.3 and 7.3 were detected inside the cell and attributed to a pH gradient of 1 pH unit over the anammoxosome membrane, where most of the ATPase was found [68]. The anammoxosome therefore is presently the only known organelle that drives ATPase by outward proton flux.

Anammoxbacteria can grow autotrophically with NO_2^-, NH_4 and CO_2. They fix CO_2 via the acetyl-CoA pathway and produce N_2 and some NO_3^- (10%) [69]. Currently the following electron pathway is proposed:

$$NO_2^- + 2H^+ + e^- \Rightarrow NO + H_2O(E_0' = 380mV)$$

$$NO + NH_4^+ + 2H^+ + 3e^- \Rightarrow N_2H_4 + H_2O(E_0' = +60mV/+130mV)$$

$$N_2H_4 \Rightarrow N_2 + 4H^+ + 4e^- E_0' = -750mV.$$

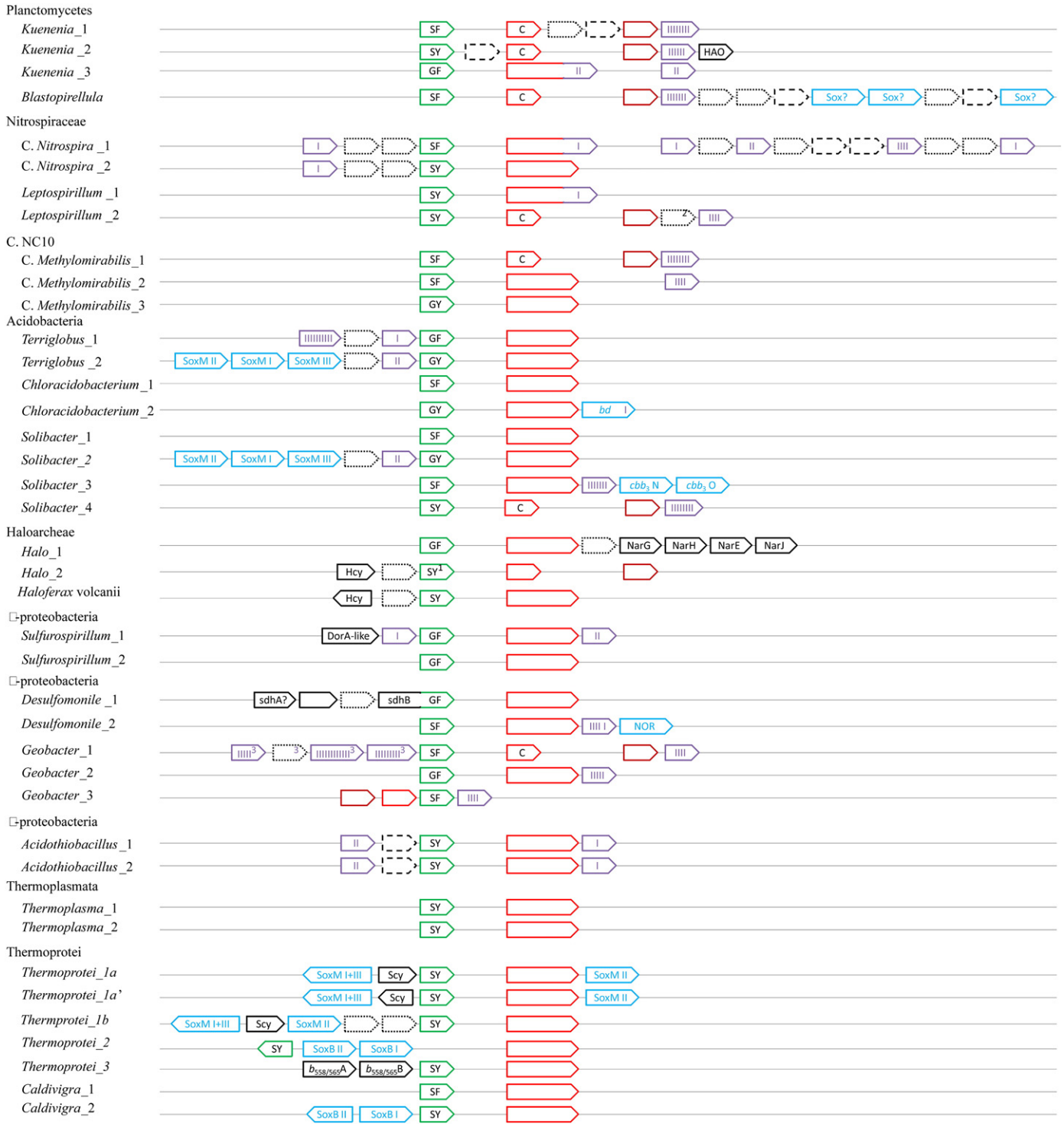


Fig. 4. A schematic representation of the genomic context of the Rieske/cyt*b* complex in species that have multiple copies of the enzyme. Genes coding for the Rieske protein, cytochrome *b*, *c*-type cytochromes and further enzymes involved in bioenergetic reaction chains are depicted. The arrow indicates the 'sens of lecture' of DNA. In the arrow representing the gene coding for the Rieske protein (green) two letters symbolize the nature of the two amino acid residues that seem to determine the redox midpoint potential of the cluster. A 'C' written in the arrow of the cytochrome b_6 genes (red) symbolizes the presence of a cysteine residue, potential ligand to a heme c_i. Subunit IV is depicted by a dark-red arrow and unsplit cytochrome *b* by a long red arrow. In the arrows representing genes coding for *c*-type cytochromes (violet), each vertical bar symbolizes a CxxCH motif. For the oxidases (blue) the type of the oxidase and the subunit are indicated. Additional proteins implicated in bioenergetics are symbolized by black arrows. Dotted arrows stand for hypothetical proteins and dashed arrows for proteins not involved in bioenergetics. Halo_1 and Halo_2 stands for the two Rieske/cyt*b* complexes from *Halorhabdus utahensis*, *Haloarcula marismortui*, *Halomicrobium mukohataei*, *Haloferax volcanii*, *Halogeometricum borinquense*, *Halorubrum lacusprofundi*. [1]*Halogeometricum borinquense* and *Halorubrum lacusprofundi* do not have the Rieske protein in this position. It is encoded somewhere else in the genome. [2]Not present in *Leptospirillum ferrooxidans*. Geobacter_1 is present in *Geobacter daltonii*, *Geobacter sp.* M21, *Geobacter sp.* M18, *Geobacter bemidjiensis* and *Geobacter uraniireducens*. Geobacter_2 is present in *Geobacter metallireducens*, *Geobacter uraniireducens*, *Geobacter bemidjiensis*, *Geobacter sp.* M18 and *Geobacter sp.* M21. Geobacter_3 is present in *Geobacter daltonii* and *Geobacter metallireducens*. [3]These proteins are present in *Geobacter bemidjiensis* only. Thermoprotei_1a is present in *Metallosphaera sedula* and *Metallosphaera cuprina*, Thermoprotei_1a′ in *Sulfolobus acidocaldarius*, *Sulfolobus solfataricus* and *Sulfolobus islandicus*, Thermoprotei_1b in *Sulfolobus tokodaii*. Thermoprotei_2 and _3 are present in *Sulfolobus tokodaii*, *Sulfolobus islandicus*, *Sulfolobus solfataricus*, *Sulfolobus acidocaldarius*, *Metallosphaera sedula* and *Metallosphaera cuprina*. In *Sulfolobus islandicus* the presence of cyt$b_{558/565}$AB in genomic context with the Rieske/cyt*b* complex depends on the strain. Scy stands for sulfocyanin, Hcy for Halocyanin, HAO for hydroxylamine oxidase, NOR for Nitric oxide reductase, sdh for complex II, Dor for DMSO reductase, Nar for nitrate reductase.

The redox potential -span between electrons from hydrazine oxidation and those needed for nitrite or ammonia and NO-reduction is largely sufficient to drive proton transfer across the membrane but the energetics of the reactions are not yet clear and many different schemes have been put forward [35,67,70–74]. In general, electrons from N_2H_4 oxidation (−750 mV) are proposed to join the quinone pool *via* an unknown mechanism; electrons from the quinone pool are proposed to be delivered back to the interior of the anammoxosome by a Rieske/cyt*b* complex for nitrite oxidation and hydrazine formation.

The three Rieske/cyt*b* complexes of *Kuenenia* have been shown by proteomic studies to be expressed, one of them predominantly [71]. The latter features a gene coding for a hydrazine oxidase that follows immediately the genes coding for one Rieske/cyt*b* complex in *K. stuttgartiensis* (Fig. 4, [70]).

Electrons from hydrazine oxidation are also proposed to serve the acetyl-CoA cycle for carbon fixation and are thus withdrawn from the anammox cycle. The fact that nitrite provided in the medium was converted partially (10%) to nitrate and the presence of a nitrite oxidoreductase (Nxr) in the genome prompted de Almeida [70] to propose a reverse electron flow from nitrite to quinone in order to replenish the electrons consumed by CO_2 fixation. The redox midpoint potentials of the nitrate/nitrite couple and menaquinone are at +430 mV and −70 mV, respectively, and a transmembrane potential of 60 mV as deduced from a measured pH gradient of 1 unit is far from sufficient to drive this energetically uphill reaction. A mechanism has not yet been proposed and a membrane subunit for the nitrite oxido-reductase Nxr is still elusive.

In addition to autotrophic growth on NO_2^- and NH_4, Anammoxbacteria have been proposed to metabolize other substrates such as Fe(II) [75], organic components and NH_4 as electron donors, Fe(III) [75], manganese oxides [75], NO_3^- as electron acceptors [41] and this versatility was put forward to explain the huge redundancy of respiratory genes in the genome. Indeed, about two hundred genes of the genome from *K. stuttgartiensis* code for energy metabolism, among which the three Rieske/cyt*b* complexes, four ATPases, one nitrite reductase (cd_1-NirS), nine genes coding for hydrazine oxidases (HAO) [75] and one nitrite oxidoreductase (Nxr) [36] were described.

The three Rieske/cyt*b* complexes in the genomes of *K. stuttgartiensis* and KSU-1 have three different sequence signatures in the Rieske subunit (GF, SF and SY), suggesting that they may operate under different redox conditions that could be related to different redox states of the quinone pool under various growth conditions or to a hypothetical implication in reverse electron transfer.

2.2. Candidatus phylum NC10

Candidatus Methylomirabilis oxyfera was discovered recently and described as an anaerobic methanotroph [76]. It converts methane to CO_2 by the same series of enzymes as present in aerobic methanotrophs. The O_2 necessary for the reaction is proposed to be produced from NO by a dismutation reaction catalyzed by an enzyme homologous to nitric oxide reductase (NOR). The genome [76] harbors three copies of Rieske/cyt*b* complexes, one grouping with Chlorobiaceae and the two others with the Cys-group on the phylogenetic tree. The quinone type is not yet known. Proteomic, transcriptomic [77,78] as well as isotope labeling studies [76] are available but no data have been published on expression of Rieske/cyt*b* complexes, so far. In reaction schemes a single copy of the complex is proposed to integrate the electron transfer chain and to donate electrons to a cd_1-NirS. Substrate quinols for the Rieske/cyt*b* may be reduced by complex I, itself reduced by NAD(P)H that could be produced during the formaldehyde to CO_2 transition, the final steps of the methane oxidizing reaction chain [79]. Oxidation of methanol produces electrons of low enough redox potential to reduce the quinone pool but quinone reduction by methanol dehydrogenase has not yet been shown, at variance with its sister enzyme ethanol dehydrogenase [80].

Metabolic versatility of *Candidatus M. oxyfera* has not been published and possible reasons for the presence of multiple copies of Rieske/cyt*b* complexes are presently unknown.

2.3. Acidobacteria

Acidobacteria form a heterogenous group with respect to the phylogeny of their Rieske/cyt*b* complexes. The quinone type of *Candidatus C. thermophilum* and *Granulicella* was reported to be MK-8 [81–83].

Terriglobus saanensis, reported to live microaerobically or aerobically at pH 6.5, has two Rieske/cyt*b* complexes phylogenetically situated in the Planctomycetes/Acidobacteria/Nitrospiraceae group. One of the two Rieske/cyt*b* complexes is in genomic context with a SoxM oxidase (Fig. 4). As far as we know, no data have been published on its energy metabolism.

Candidatus C. thermophilum is an aerobic and photosynthetic organism with a photosynthetic equipment similar to that of Chlorobiaceae. One of the two Rieske/cyt*b* complexes of this organism is localized basally in the phylum of Chlorobiaceae suggesting a lateral gene transfer from the ancestor of the Chlorobiaceae before the radiation of the currently known species of this phylum. In the genome of *Candidatus C. thermophilum* this complex is situated immediately upstream of subunit I of a *bd*-oxidase that features a cytochrome *c* binding motif [38] (Fig. 4). Photosynthetic activity seems to be quenched if O_2 is present and metatranscriptomic analysis results suggest metabolic changes between day and night [84] but the expression pattern of the Rieske proteins or cytochromes *b* was not included in the studies.

Solibacter usitatus has four Rieske/cyt*b* complexes. Two are phylogenetically situated in the Planctomycetes/Acidobacteria/Nitrospiraceae group and the two other in the Cys-group. None has the conserved cysteine residue. One of the complexes of the Cys-group is in genomic context with a cbb_3 oxidase whereas a SoxM oxidase is part of the genomic context of one of the complexes from the Planctomycetes/Acidobacteria/Nitrospiraceae group (Fig. 4). The genome of *S. usitatus* is huge, with its 9.9 Mbp (for comparison Terriglobus has 2.2 Mbp) and codes for many paralogous proteins [85].

All Acidobacteria discussed here also feature a gene cluster coding for an ACIII. Possible reasons for the redundancy of Rieske/cyt*b* complexes in Acidobacteria are presently unknown.

2.4. Deltaproteobacteria

Only some deltaproteobacteria have Rieske/cyt*b* complexes. One copy of this enzyme with an unsplit cytochrome *b* and a Rieske protein with a predicted low-potential 2Fe2S cluster, present in some *Geobacter* species, is located on the phylogenetic tree basal in the proteobacterial branch. All the other copies, i.e. the second and third versions present in some *Geobacter* species and the enymes from *D. tiedjei*, and the single copy from *Desulfococcus oleovorans*, *Anaeromyxobacter dehalogenans*, *Haliangium ochraceum*, *Sorangium cellulosum*, *Anaeromyxobacter sp.*, *Desulfuromonas acetoxidans* are located among the Planctomycetes/Acidobacteria/Nitrospiraceae and the Cys-group, indicating that for this phylum acquisition of the complex via lateral gene transfer is predominant.

For *D. tiedjei* little is known, it can grow by reduction of sulfur compounds and halogenated molecules and by oxidation of organic compounds. One Rieske/cyt*b* complex is in genomic context with a gene coding for a nitric oxide reductase. The second Rieske/cyt*b* complex is downstream of four genes that may code for a complex II. In the actual version of the genome the iron–sulfur binding subunit of complex II is fused to the gene coding for the Rieske protein, an isolated case that may merit further investigation (Fig. 4). Quinones have not been found, which is surprising since several quinone-using enzymes are encoded in the genome [86].

Geobacter species were reported to contain MK-8 [87]. The presence of MK is in agreement with the sequence signature of the Rieske

protein (GF, SF or GY). These bacteria grow mainly anaerobically even if *G. sulfurreducens* was reported to be able to use up to 5% of O_2 as electron acceptor [88]. They can also grow with nitrate, fumarate or some organic compounds as electron acceptors [89–94]. They contain abundant *c*-type cytochromes in the outer membrane that are involved in extracellular metal oxidation and may serve as short-time electron storage if no substrate is available [95,96]. Many *Geobacter* species have the conserved Cys residue in their cytochrome b_6 sequence, indicating a possible presence of heme c_i. Expression of Rieske/cyt*b* complex has not been found by Ahrendt and coworkers [89] under iron oxidizing conditions, however Ding and colleagues [97] observed an up-regulation of one Rieske/cyt*b* complex from *G. sulfurreducens* under Fe–citrate growth and the necessity for a proton pump in addition to complex I has been postulated under FeIII growth to cope with the acidification of the cytoplasm due to acetate oxidation [89]. Which type of enzyme fulfills this postulated pumping activity has not yet been discovered. In the genomic context of all Rieske/cyt*b* complexes from *Geobacter* species a multi-heme (four or five hemes) cytochrome *c* is present, in most cases situated downstream of cytochrome *b* (Fig. 4). *Geobacter metallireduces* and *Geobacter daltonii* are to our knowledge the only organisms known that inversed the canonical order of genes from Rieske/cyt*b* to cytb_6/SUIV/Rieske. Many *Geobacter* species feature also the genes coding for an ACIII in their genome. Possible reasons for the redundancy of Rieske/cyt*b* complexes in Deltaproteobacteria are presently elusive.

2.5. Epsilonproteobacteria

Among the epsilonproteobacteria only *Sulfospirillum deleyianum* was found to encode more than one Rieske/cyt*b* complex. This species grows microaerobically or aerobically with various electron donors (H_2, formate, fumarate, pyruvate) and electron acceptors (nitrate, nitrite, sulfite, thiosulphate, sulfur, DMSO, fumarate, malate, aspartate, AsV, MnIV, and FeIII). FeIII-hydrite reduction to ferrous minerals was described to be mediated by sulfur compounds [98].

One of the Rieske/cyt*b* complexes is preceded in the genome by a mono-heme cytochrome *c* and immediately followed by a diheme cytochrome *c*, the other one does not have *c*-type cytochromes in genomic context (Fig. 4). On the tree, the complex without cytochrome *c* is localized at the base of the epsilonproteobacterial group, the one with two cytochrome *c* at the base of a group comprising epsilonproteobacteria and complexes from Aquificales that have acquired the enzyme by lateral gene transfer. Nothing is known about the functional implications of the two Rieske/cyt*b* complexes of *Sulfurospirillum*.

2.6. Gammaproteobacteria

Acidithiobacillus ferrooxidans and *ferrivorans* have two closely related Rieske/cyt*b* complexes, indicating that gene duplication took place in the ancestor of *Acidithiobacilli*. The quinone content was determined for *Acidithiobacillus caldus* to be UQ [99].

A. ferrooxidans was the subject of several studies investigating the role of the two Rieske/cyt*b* complexes [27,46,100]. It has been shown that in this strictly aerobic, acidophilic organism both Rieske/cyt*b* complexes are expressed under FeII growth conditions, whereas only one is expressed when growth is sustained by thiosulfate [27]. Inhibition of O_2-consumption with thiosulfate as a reductant by the typical bc_1 complex inhibitors myxothiazol and stigmatellin has been interpreted as a participation of a Rieske/cyt*b* complex in the electron transfer chain to oxygen [100]. FeII oxidation is inhibited by uncouplers that abolish the membrane potential and also by complex I inhibitors, indicating that a membrane potential is necessary for this process [101]. A reversed electron flow from cytochrome *c* to the quinone pool via a Rieske/cyt*b* complex was consequently proposed to be part of FeII metabolism. Redox midpoint potentials of the cofactors of the Rieske/cyt*b* complex expressed under FeII growth conditions were determined to +490 mV at pH 4 for the Rieske protein [47], +20 mV for one of the *b*-hemes at all pH values investigated and −170/+20 mV for the other *b*-heme at pH 7.4/3.5 [46]. UQ was supposed to sense the external pH and to work at a midpoint potential of +320 mV at the Q_o site at pH 4 but of +80 mV at the Q_i site exposed to pH 7. This redox potential difference was proposed to drive reverse electron transfer [45]. A characterization of the second Rieske/cyt*b* complex from *Acidithiobacillus* is not yet published and we therefore do not know what differentiates the two complexes and governs their specificity with respect to directionality of the reactions. It is noteworthy that alpha- and betaproteobacteria live without low potential substrates able to reduce their NAD^+ pool and thus obviously can operate their bc_1 complex in forward and reverse electron transfer without needing two copies of the enzyme to do so. In these cases all reactions were shown to be reversible and directionality seems to be governed by ambient redox potential, pH and membrane potential [102–104].

2.7. Nitrospiraceae

A much less studied group of acidophilic organisms that also lives aerobically and autotrophically by FeII-oxidation are the Leptospirillaceae. Their quinone type has not yet been determined. Similar to *Acidithiobacillus* they also exhibit two Rieske/cyt*b* complexes and were reported to have an acetyl-CoA pathway for CO_2 fixation [37]. Among their two Rieske/cyt*b* complexes, one features a split cytochrome *b* and a cysteine residue at the same sequence position as that covalently ligating heme c_i in cyanobacteria and chloroplasts. A tetraheme *c*-type cytochrome is present in the genomic context. In the second Rieske/cyt*b* complex, a mono-heme *c*-type cytochrome is fused to the C-terminus of cytochrome *b* [33] (Fig. 4). In analogy to the results obtained on *Acidithiobacillus*, reversed electron transfer is supposed to be part of the bioenergetic system of Leptospirillaceae. For *Leptospirillum ferrooxidans*, FeII was reported to be the only electron donor sustaining growth [105]. The presence of a second Rieske/cyt*b* complex, however, indicates that alternative metabolisms may be operating, as supported by the presence of two gene clusters coding for sulfide–quinone-reductases in the genome of *Leptospirillum ferrodiazotrophum* [37]. It is not known so far whether the two complexes are specialized in forward and reverse electron transfer and if so which might be the one involved in reverse electron flow. The possible presence of heme c_i in one of the two complexes makes these organisms interesting species for the study of this co-factor.

Also among the Nitrospiraceae but with a different metabolism is *Candidatus N. defluvii*. Its quinone type is not known. It lives as an autotrophic aerobic nitrifier transforming ammonium to nitrate via nitrite [106]. Nitrite to nitrate conversion was shown to be catalyzed by Nxr, an enzyme that was proposed to transfer electrons to quinones. A membrane attached, quinone interacting subunit and a mechanism that allows for this uphill electron transfer (the NO_2^-/NO_3^- couple is at +430 mV, quinones at +100 mV or at −70 mV, depending on the quinone type), however, are still elusive. Nxr may also transfer electrons to cytochrome *c* that injects them in reverse electron flow through a Rieske/cyt*b* complex into the quinone pool, but the energetics of this pathway is not better understood either [36]. Two Rieske/cyt*b* complexes are encoded in the genome. Both are phylogenetically close to the complex from *Leptospirillum* in the Planctomycetes/Acidobacteria/Nitrospiraceae-group. An unusual *bd*-oxidase with a predicted CuA site is also present in the genome that may accept electrons from periplasmic electron carriers, reduced by forward electron transfer through a Rieske/cyt*b* complex rather than from quinols directly [36].

2.8. Haloarchaea

Haloarchaea are the only organisms found so far that acquired Rieske/cyt*b* complexes via interdomain lateral gene transfer (for a recent review see [1]). Many of them encode two Rieske/cyt*b* complexes

in their genomes; one is found in genetic context with nitrate reductase genes and has its closest relatives among Actinobacteria and the *Thermus*/*Deinococcus* group. It has been proposed to function in nitrate respiration [107–110]. The second Rieske/cyt*b* complex of Haloarchaea (which is the only one for some species not listed here) features a split cytochrome *b* sequence as do cyanobacteria, chloroplasts, Heliobacteria and Bacilli but does not feature the conserved cysteine residue. Haloarchaea apparently have acquired this complex from an ancestor of the above mentioned phyla. This complex is in genomic context with a gene coding for halocyanin, a copper protein and was suggested to be involved in electron transfer towards oxygen, but experimental data on this subject are completely absent. The Rieske subunit of this enzyme has a sequence signature that suggests it to be of high redox potential, which is in apparent contradiction to the fact that MK is the only quinone species that has been found in these organisms [23,111]. However, these organisms live at high salt concentrations and an influence of ionic strength on the redox potential remains to be investigated. High salt concentration in the medium and inside the cell [112] should raise the redox midpoint potential of quinones (by 60 mV for 4 M NaCl) if the quinone molecule in the binding site senses the full ionic strength. For the Rieske cluster, the shift in its redox potential is more difficult to predict and has to await experimental results.

2.9. Thermoprotei

Thermoprotei are acidophilic organisms that grow at elevated temperature (pH 4 and 85 °C for *Caldivirga maquilingensis* [113]). A proton gradient resulting in a proton motive force of 180 mV was observed for *Sulfolobus acidocaldarius* [114].

Quite a number of articles have been published on bioenergetic enzymes from *Sulfolobus* and a few papers on *Metallosphaera*. *Sulfolobus* represents a special case among organisms with multiple copies of Rieske/cyt*b* complexes, since for this organism many biochemical and biophysical results do exist. Most were obtained before the genomes of these Archaea were sequenced. Reading through the literature, we tried to correlate available experimental data and sequence information. We are convinced that resuming experimental studies of these organisms, now, with the available genome information at hand, is a worthwhile task.

On a phylogenetic tree of cytochrome *b*, the three complexes encoded in the genomes of *Sulfolobus* and *Metallosphaera* species cluster in three different parts of the archaeal part of the tree (Fig. 3). One group is formed by a Rieske/cyt*b* complex in genomic context with SUII of SoxM oxidase, SUI and III (fused) are encoded on the opposite strand (Fig. 4). This SoxM bb_3 oxidase from *S. acidocaldarius* has been purified together with the corresponding Rieske protein (SoxF) and cytochrome *b* (SoxG) as a catalytically competent complex [115]. Its expression was reported to be up-regulated under growth on yeast extract compared to growth on sulfate in *Metallosphaera sedula* [32].

A second group is composed of cytochrome *b* sequences that have SUI and II for SoxB oxidase in genomic context with the Rieske protein encoded next to them on the opposite strand (Fig. 4). This complex has so far been purified with the SoxB aa_3-oxidase and cytochrome *b* (SoxC) and is reported to have quinol oxidase activity [49,54]. SoxB is assumed to be a quinol oxidase since no CuA center was found [2]. A Rieske protein has not been detected in the isolated preparation. When integrated in lipid vesicles, the purified complex produces a proton gradient with artificial electron donors [49]. The Rieske protein from the third group (see below) has been purified and reconstituted with this preparation which allowed reduction of SoxB by exogenous cytochrome *c* and a proton pump activity of $1.2H^+/e^-$ was measured [49]. In membranes obtained from cells expressing SoxB, reduction of a Rieske protein by addition of succinate in the presence of cyanide could be observed [116]. Taken together these data indicate that SoxB indeed forms a complex with a cytochrome *b* and a Rieske protein. The predicted quinol oxidase nature of SoxB may warrant reinvestigation since it is difficult to imagine how a quinol oxidase can interact with a Rieske/cyt*b* complex. In *M. sedula*, gene expression of the SoxB cluster is up-regulated when grown on sulfur [32].

The hemes of cytochromes "*b*" of both above described Rieske/cyt*b* complexes are in fact hemes A_s [55]. Whereas heme-copper oxidases can be found with a variety of different heme-types the presence of a heme other than heme *b* in a Rieske/cyt*b* has been reported for Crenarchaea only.

A third Rieske/cyt*b* complex present in *Sulfolobus* and *Metallosphaera* features genes for a Rieske protein and for cytochrome *b* preceded by genes coding for CsbA and B. CsbA is a membrane attached mobile [117] mono-heme cytochrome *b* that could function as an electron shuttle between the Rieske protein and a terminal electron acceptor enzyme and CsbB is a highly hydrophobic protein of so far unknown function [118]. In *M. sedula* the expression of CsbA and B is up-regulated during growth on $FeSO_4$ and FeS [32], and in *S. acidocaldarius* under low O_2 tensions [118]. Two different promoters for the genes coding for the Rieske protein (SoxL) and cytochrome *b* (SoxF) and for those coding for CsbA and B have been observed in *Metallosphaera* [32]. In *Sulfolobus*, however, mRNA coding for all four proteins has been detected as well as mRNA coding for the Rieske protein (SoxL) on its own [119].

The pK value of a Rieske protein from *S. acidocaldarius* has been determined to be at 6, which is about 2 pH units below the pK values determined for neutrophilic species, in agreement with the acidophilic nature of the organisms [47,120]. Another study observed a pK of 4 for the Rieske protein in genomic context with CsbA and B and an E_m shift around pH 5.4 for the Rieske protein in genomic context with SoxM [56]. The redox midpoint potential of the Rieske cluster was found around 350 mV to 390 mV at pH 7 [56,120] as expected for the sequence signature (SY) and the presence of high potential quinone.

Schmidt [57] determined that 1.7 to 2 Rieske proteins per SoxM are present in membranes under all growth conditions, indicating that more than one Rieske/cyt*b* complex is expressed.

The phylogenetic tree of Crenarchaea indicates that gene duplication of the Rieske/cyt*b* complex took place early in the evolution of Archaea. Genomic association of the complex with a SoxM or a SoxB oxidase or CsbA/B proteins apparently occurred subsequently and before speciation of *Sulfolobus* and *Metallosphaera* and a tight interaction of the Rieske/cyt*b* complexes with their reaction partners may be a reason for the stabilization of multiple copies of the enzyme in these genomes.

A curiosity is the gene Msed_1191, a fusion of cytochrome b_6 (with five transmembrane helices) and a Rieske protein. It is highly transcribed under growth with $FeSO_4$ and has so far no equivalent outside *Metallosphaerae* [121].

2.10. Thermoplasmata

Little literature is available on Thermoplasmata. Their two Rieske/cyt*b* complexes are localized on a phylogenetic tree among the Crenarchaea, suggesting that Thermoplasmata acquired their Rieske/cyt*b* complexes via lateral gene transfers as suggested for several other genes from *Thermoplasma acidophilum* [122]. Menaquinone [123], methylthionaphthoquinone and thermoplasmaquinone [16] were found in equal amount in aerobically grown organisms, whereas TPQ was largely predominant under anaerobic conditions [124]. The redox midpoint potential of TPQ and MTK are still unknown, that of MK is in apparent contradiction to the predicted high redox potential of the Rieske cluster. No other bioenergetic proteins were found in the genomic context of the two Rieske and cytochrome *b* genes. This and the absence of information about the redox midpoint potentials for TPQ and MTK precludes for the time being any hypothesis for specialized functions of the two Rieske/cyt*b* complexes.

3. Conclusions and perspectives

Multiple copies of Rieske/cyt*b* complexes in a single organism occur in species that are located all over the phylogenetic tree. Unfortunately, most of these organisms are actually poorly studied and often difficult to cultivate which limits available information. The here presented overview allows to put forward a few hypotheses for the existence of multiple copies of the enzyme and to propose experiments to test them:

Firstly, Fe(II) reducing, acidophilic bacteria seem to employ one Rieske/cyt*b* complex in reverse electron flow and a second one in forward electron flow. The molecular bases for this surprising bias in directionality are still unknown and could be addressed by a comparative study of the two complexes from either *Leptospirillum* or *Acidithiobacillus*.

Secondly, in Thermoprotei, Rieske/cyt*b* complexes were found to form a complex with oxidases that are expressed under different growth conditions. The tight association of the two catalytic units may have induced a duplication and specialized adaptation of the Rieske/cyt*b* modules to the different oxidase counterparts. Microbiological and biochemical studies on Thermoprotei are already available and resuming them in the light of the genome sequences is certainly a rewarding task.

Thirdly, several organisms seem to have acquired a second Rieske/cyt*b* complex from organisms of the Cys-group. The conserved cysteine residue that covalently links heme c_i to the protein in the so-far studied species indicates that they may possess this heme cofactor. We may therefore speculate that a property of the complex that is linked to the presence of this heme conferred the evolutionary advantage to the organisms that resulted in the stabilization of the genes coding for a second Rieske/cyt*b* complex in their genomes. Our survey of available (scarce) information on organisms containing the conserved cysteine residue in their cytochrome b_6 sequence did not yet allow to come up with any correlation between the lifestyle of the respective organisms and the putative presence of heme c_i. Further investigations are badly needed and a study of the presence and properties of heme c_i in the Rieske/cyt*b* complexes of *Leptospirilla*, *Geobacter* or Planctomycetes may contribute to resolve the enigma of the function of heme c_i.

Whereas in most organisms the predicted redox midpoint potential of the Rieske cluster is adapted to the redox potential of the quinone pool (see Fig. 2), in some species menaquinones coexist with a Rieske protein with a predicted high redox potential (sequence signature SY). Experimental results on redox reactions of a bc_1 complex harboring a high potential Rieske cluster with low potential quinones (namely in this study rhodoquinone, [125]) resulted in a strongly reduced efficiency of the enzyme due to numerous by-pass reactions in the Q_o site, including radical oxygen species production. Therefore, the occurrence of predicted high potential Rieske clusters in menaquinone containing anaerobic Anammoxbacteria but also aerobic Haloarchaea (and potentially *Solibacter*) calls for further studies. To ensure an efficient bifurcated quinol oxidation at the Q_o side under these conditions we can think of two possibilities: it may turn out that the prediction of the redox midpoint potential, based on a correlation between the nature of two specific residues close to the cluster binding site and the redox midpoint potential of the cluster so far determined from various organism and mutants [19–22] will not hold for a wider sample of organisms. This point should be addressed experimentally by redox-titrating Rieske proteins from phylogenetically distant organisms. Alternatively, the Q_o site of the respective proteins may raise the quinone redox potential, implementing a stronger affinity for quinol over quinone. The sensitivity of the EPR signature of the Rieske cluster towards the occupancy of the Q_o-site [126] is a valuable tool to address this issue experimentally.

In Haloarchaea the predicted redox midpoint potentials of the Rieske cluster and the quinones may vary from the operating potentials due to the high salt concentrations that the organisms require, as discussed above.

It is noteworthy that different derivatives of benzo- and naphthoquinones with yet undetermined redox midpoint potentials have been found in various organisms (such as Sulfolobusquinone, Thermoplasmaquinone and Methylthionaphthoquinone). Knowledge of their redox midpoint potentials will be important for our understanding of the bioenergetic reaction chains of the corresponding organisms.

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