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The bioenergetic role of dioxygen and the terminal oxidase(s) in cyanobacteria

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Abstract

Owing to the release of 13 largely or totally sequenced cyanobacterial genomes (see http://www.kazusa.or.jp/cyano and www.jgi.doe.gov/), it is now possible to critically assess and compare the most neglected aspect of cyanobacterial physiology, i.e., cyanobacterial respiration, also on the grounds of pure molecular biology (gene sequences). While there is little doubt that cyanobacteria (blue-green algae) do form the largest, most diversified and in both evolutionary and ecological respects most significant group of (micro)organisms on our earth, and that what renders our blue planet earth to what it is, viz. the O_2-containing atmosphere, dates back to the oxygenic photosynthetic activity of primordial cyanobacteria about 3.2×10^9 years ago, there is still an amazing lack of knowledge on the second half of bioenergetic oxygen metabolism in cyanobacteria, on (aerobic) respiration. Thus, the purpose of this review is threefold: (1) to point out the unprecedented role of the cyanobacteria for maintaining the delicate steady state of our terrestrial biosphere and atmosphere through a major contribution to the poising of oxygenic photosynthesis against aerobic respiration ("the global biological oxygen cycle"); (2) to briefly highlight the membrane-bound electron-transport assemblies of respiration and photosynthesis in the unique two-membrane system of cyanobacteria (comprising cytoplasmic membrane and intracytoplasmic or thylakoid membranes, without obvious anastomoses between them); and (3) to critically compare the (deduced) amino acid sequences of the multitude of hypothetical terminal oxidases in the nine fully sequenced cyanobacterial species plus four additional species where at least the terminal oxidases were sequenced. These will then be compared with sequences of other proton-pumping haem–copper oxidases, with special emphasis on possible mechanisms of electron and proton transfer.

Keywords: Respiration; Photosynthesis; Oxygen; Terminal oxidase; Proton-pumping; Cyanobacteria; Evolution

1. Introduction: molecular oxygen, a devil in disguise?

The first few lines may be devoted to molecular oxygen as such, the key player in our game. Though fairly ubiquitous on, and absolutely characteristic of, our Blue Planet earth molecular oxygen, i.e., oxygen gas (O_2), is the strangest and most mysterious gas known here. The cosmic (solar) abundance of the element O, with H:He:O:C:N:-others = 93.93:5.93:0.07:0.04:0.01:0.02 (atom%) in the average matter of stars [1], is even higher than that of other heavier elements, due to the important "catalytic" role of ^{15}O in the Bethe–Weizsäcker (or C–N–O) cycle for the generation of He from H in stars at $>15\times10^6$ °C. However, oxygen gas, the unique biological by-product of oxygenic,

Abbreviations: CcO, cytochrome *c* oxidase; C_i, inorganic carbon; CM, cytoplasmic (plasma) membrane(s); Cyt, cytochrome; Fd, ferredoxin; FeCy, ferricyanide; FNR, ferredoxin: NADP-oxidoreductase; FR, fumarate reductase; ICM, intracytoplasmic (thylakoid) membrane(s); ICP-AES, inductively coupled plasma atomic emission spectroscopy; IEP, (pH of) isoelectric point; LCAO, linear combination of atomic orbitals; PC, plastocyanin; PCC, Pasteur Culture Collection; PET, photosynthetic electron transport; P_i, inorganic phosphate; PQ, plastoquinone; QOX, quinol oxidase; RET, respiratory electron transport; ROS, reactive oxygen species; SDH, succinate dehydrogenase; TRO, terminal respiratory oxidase

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plant-type photosynthesis [2–4] (see Eq. (1)), appears to be practically inexistent in our universe as also witnessed by the absence of O_2 from volcanic exhalations, from the interior of extraterrestrial matter (comets, meteorites, etc.), and from all (terrestrial) planets known and tested so far (whereby it should be noted that simply the future search for terrestrial planets alone is facing major unexpected theoretical problems, see http://planetquest.jpl.nasa.gov/).

In contrast to all other diatomic gases such as N_2 or H_2, the energetic ground state of O_2 is not a singlet, but a triplet state, i.e., spin multiplicity is 3 (Fig. 1). According to the well-known laws of quantum mechanics (Hund's rule, Pauli principle, etc.), this means that O_2 is the only known paramagnetic diatomic gas displaying a resulting spin moment of S=1 $h/2\pi$, hence a spin multiplicity ($2S$+1) of 3 (triplet). In normal molecules, quantum mechanically treated according to the *LCAO* method, only excited states are triplet states while in O_2 the *first singlet* state uniquely lies almost 96 kJ/mol above the *triplet* ground state ([5,6]; see Fig. 1). Together with the high positive standard redox potential of O_2 of +820 mV at pH 7, this fact has the following paradoxical, yet biosphere-rescuing consequences:

Thermodynamically, on one hand, O_2 would oxidize (literally: *devour*) everything that could donate electrons to it, which, in particular, are practically all components of our biosphere including N_2. So we should be happy that nature has not yet found a catalyst (enzyme) to facilitate this (and other) reactions involving O_2 ("combustions")! There is almost no chemical compound (particularly none of biological relevance) with a redox potential more positive than O_2. The only partners that O_2 could encounter on our earth

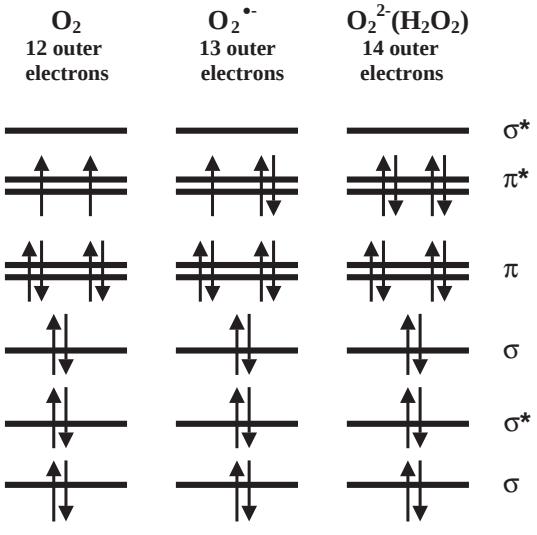


Fig. 1. Oxygen uniquely is a paramagnetic (triplet) diatomic gas whose ready reaction with usual singlet-state molecules, even if thermodynamically highly favoured, is quantum-mechanically forbidden. The figure shows the assignment of electrons to molecular orbitals resulting from LCAO treatment (Linear Combination of Atomic Orbitals) for dioxygen, the superoxide radical anion and hydrogen peroxide.

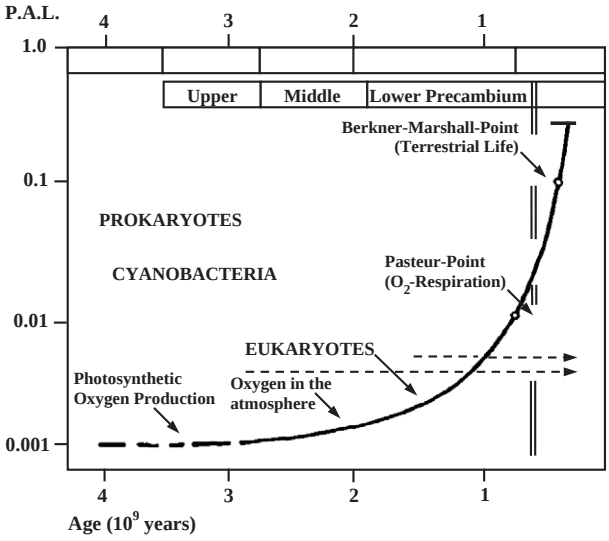


Fig. 2. The oxygen content of the earth's atmosphere during the previous 4 billion years. P.A.L., present atmospheric level, i.e., fractions of 21% v/v. The Pasteur point marks the time when the global efficiency of respiration (in terms of ATP production), because of increasing availability of O_2, exceeded that of fermentation. The Berkner–Marshall point marks the time when the ozone layer in the upper atmosphere started to prevent deleterious ionizing radiation from the outer space from reaching the surface of the earth. The transient overshoot of O_2 concentration peaking at 35% (v/v) during the carboniferous period about 300 million years ago [7] (not shown in the figure) was due to the massive development of terrestrial (in particular fern) plants under the shielding ozone layer in the upper atmosphere. It is the time to which all our present fossil fuel deposits date back, and the overshooting O_2 at first could not be compensated for by a likewise rapid development of animals. The figure is compiled from data in [7,14,30,211–213].

are prompt and ready electron donors (reductants). Yet, *kinetically* almost all of those reactions, being reactions between a triplet-state (*paramagnetic*) molecule (O_2 as the electron acceptor = oxidant) and singlet-state (*diamagnetic)* electron donors, are so-called forbidden reactions due to the quantum mechanical law of the *forbidden spin inversion.* Thus, we owe the *peaceful co-existence* with O_2 amidst an easily oxidizable biosphere (i.e., the inherently *metastable state* of our existence) to the purely accidental (and extremely rare) combination of high *thermodynamic* reactivity with a very low *kinetic* probability of the reaction due to the forbidden spin inversion. On the other hand, it is just this delicate combination of seemingly contradictory properties that has rendered O_2 the motor of biological evolution on earth ([7] and Fig. 2). By the way, the same conflict between thermodynamical driving force and quantum mechanical barriers explains why the Fe^{2+} of haemoglobin, $E^{o\prime}$ (Hb-Fe^{3+}/Hb-Fe^{2+})=+170 mV, patiently carrying O_2 ($E^{o\prime}$=+820 mV) on its shoulder in the blood stream, is not at all oxidized in spite of the high driving force of the reaction corresponding to a $\Delta G^{o\prime}$=62.7 kJ/mol at pH 7.

It is a big privilege of living matter that, in the course of evolution, biochemical (i.e., enzymatic) methods have been developed to exploit oxygen's high reactivity as a driving force for efficient *biological energy conversion* as

the necessary counterpoise to neutralize the increase of *information* (=decrease of *entropy*) as reflected by more and more highly developed organisms, according to an irreducible law of nature, the fundamental Second Law of Thermodynamics. Unfortunately, yet characteristic of the ambivalent function of O_2 in higher organisms, it is also inherently responsible for the disagreeable process of aging (see, e.g., [7,8]). Interestingly, on the other hand, in our contemporary and basically oxic biosphere still organisms exist in O_2-free ecological niches that are irreversibly damaged by even low concentrations of O_2. Such obligate anaerobiosis, as exemplified by the methanogens or by certain *Clostridia*, has received much attention during the decades [9–11]. It is noteworthy that even aerobic (mitochondrial) respiration is, at its roots, still an anaerobic process as it presupposes the whole (anaerobic) glycolytic sequence and comprises only CcO as the very last, necessarily aerobic step. Clearly, the large number of extant anaerobic forms of bacterial respiration nicely document that the quintessence of respiration is not oxygen, but membrane-bound electron transport from "low-potential" donor (large negative redox potential of an electropositive compound) to "high-potential" acceptor molecules (high positive redox potential of an electronegative compound) coupled to chemiosmotic ATP synthesis [12,13].

Conforming to this scenario is the suggestion that biological electron transport, beside having a common (monophyletic) origin as formulated by the conversion hypothesis [14,15], has evolved from dark anaerobic and chemiosmotic transmembrane electron transfer [16] (prerespiratory electron transport [17]), via anoxygenic and oxygenic photosynthesis to aerobic respiration (see [18]). At any rate, and conforming to the above-mentioned properties of O_2, it seems logical that the final and mechanistically fairly easy conversion of preexisting photosynthetic electron flow chains into full-fledged (aerobic) respiratory chains may have been preceded by a more or less extended period of mere detoxification of O_2 and its even more dangerous partially reduced intermediates (ROS) through enzymes such as superoxide dismutases, catalases, peroxidases, peroxiredoxins, etc. Again, the primordial cyanobacteria would be the first candidates to elaborate such O_2- and ROS-detoxifying enzyme systems as they clearly were challenged first by O_2 within themselves. Indeed, we have isolated and characterized a wide variety of such enzymes from cyanobacteria [19–27], thereby strengthening the concept of cyanobacteria as the tamers of O_2 (see the next section).

With respect to respiration, it must be remarked that the so-called "respiration-early" hypothesis [28], a mere revival of Egami's nitrate respiration hypothesis [29], faces the problem that oxidized nitrogen clearly was not present in the more or less reducing environments of the early earth [30] and a few molecules of NO here and there in the biosphere [31] certainly would not have been sufficient to "taper" evolution into the complicated molecular scaffolds of a full-fledged terminal oxidase. The striking similarity of certain terminal respiratory enzymes involved in "oxygen respiration" with those of "nitrate respiration" [32,33] is no bias for the *direction* of evolution, from oxy-N to O_2 or vice versa—but imagine the origin of human language in a throat without vocal chords. . .?

2. Cyanobacteria—the tamers of oxygen?

Yet, returning to the origin of life and its evolution (Fig. 2), it is imperative to recall that beyond any doubt the first steps in the evolution of living matter ("cells") must have been taken in the absence of both ionizing radiation and free oxygen. These two forms of high-energy impact would necessarily have acted as lethal weapons on the delicate and vulnerable constituents and structures of an evolving living cell (protocell, eobiont, coacervate, etc.). Until recently, the heterotrophic primeval soup scenario of Oparin and Haldane (see [14]), presupposing the substantial quantities of highly organized reduced organic substances (up to porphyrins) that Urey and Miller [34] could abiotically have provided for, had been the generally accepted, though highly speculative hypothesis for the origin of life on earth. But now we have, as a competing hypothesis, though no less speculative, the autotrophic redox scenario of Wächtershäuser [35,36], which even pretends to be capable of explaining the origin of the first living cell [37].

Without any doubt, the most decisive factor in the evolution of life on earth was free oxygen (O_2), significant quantities of which were first set free through coordinated biological photooxidation of water by the primordial cyanobacteria 3.2×10^9 years ago [38–41]. Fig. 2 follows the evolution of the oxygen budget of our biosphere and atmosphere during the aeons. The pivotal role of the cyanobacteria is evident. Particularly note that free oxygen, stemming from cyanobacteria, was an essential prerequisite for the occurrence of simple eukaryotic cells already [7], which is still witnessed by the fact that no primarily (obligately) anaerobic eukaryotic organism is known even in our contemporary biosphere.

The cycling of O_2 between the three main terrestrial reservoirs (H_2O, CO_2, and O_2) in the atmosphere and hydrosphere is best reflected by Eq. (1), a fairly simple (bioenergetic) equation simultaneously describing oxygenic, plant-type photosynthesis (from left to right) and aerobic respiration (from right to left). On the basis of exchange rates and reservoir pool sizes it is calculated that the mean residence time ("half-life") of oxygen is 6000 years in atmospheric O_2, 500 years in CO_2 (including dissolved (bi)-carbonate), and 20 million years in H_2O [14,30]. Note that it is Eq. (1), first established by the primordial cyanobacteria, that still ensures the delicate balance between the biological appearance and disappearance of O_2 in our biosphere, thus

maintaining the present 21% (v/v) O_2 in the atmosphere which we all critically depend on:

$$6CO_2 + 12H_2O \leftrightarrows C_6H_{12}O_6 + 6O_2 + 6H_2O \quad \Delta G^{o\prime} = \pm 2821.5 kJ/mol \tag{1}$$

Eq. (1) describes the present terrestrial steady state established by the concurrent and equally efficient actions of oxygenic (plant-type) photosynthesis and aerobic respiration. The equation is based on the famous Van Niel equation for autotrophic CO_2 fixation. In the endergonic direction (+2821.5 kJ/mol), exemplifying the photosynthetic primary production of biomass (start of terrestrial food chains), the process is energetically driven by light. Chemosynthetic primary production is without any significant quantitative ecological impact [42]. An estimated 10^{11} tons of carbon (in the form of CO_2) per year is converted into biomass by plant-type photosynthesis and the equivalent amount of O_2 is thereby released from water according to Eq. (1). Recent estimates assign between 20% and 30% of this worldwide primary productivity to cyanobacteria, in particular to small unicellular marine *Synechococcus* species [43] and likewise unicellular planktonic Prochlorophytes [44], which, though not especially concentrated anywhere in the euphotic zone of the oceans, are nevertheless extremely widespread in all oceans. Also see [45,46] for further evolutionary considerations.

Owing to the unprecedented protagonistic role of the cyanobacteria with respect to oxygen metabolism, being the only prokaryotic organisms combining both oxygenic photosynthesis (release of O_2) and aerobic respiration (uptake of O_2) in a single bacterial cell, some words must be devoted to the membrane-bound electron transport systems of respiration and photosynthesis in this extremely large and diversified group of organisms. Though occupying the cradle of terrestrial O_2, only half of the cyanobacterial oxygen metabolism has so far been taken seriously by the bioenergetic community, viz. oxygenic, plant-type photosynthesis. For the latter, cyanobacteria truly represent kind of godparents particularly as regards the new era of biocrystallography: the high-resolution 3D structures of no less than three most important of the transmembrane protein complexes of oxygenic photosynthesis, viz. Photosystem II [47,48], Photosystem I [49,50], and the b_6f complex [51] were obtained on *cyanobacterial* complexes (also see [52]). The other half of bioenergetic oxygen metabolism in cyanobacteria, viz. aerobic respiration, has up to now been dreadfully neglected, as is also reflected by the disgracefully poor number of major review articles available on this subject specifically [53–55]. Mostly, photosynthesis and respiration has been reviewed together [45,56,57], which obviously stems from the fact that, traditionally, while a physiologically significant role for cyanobacterial respiration had originally been denied at all [58], both PET and RET had mistakenly been localized together exclusively on ICM using cytochemistry [59] (however, see [60,63,64]). Similarly, and unfortunately, Murata and associates initially were unable to find any respiratory activity or *a*-type cytochrome in their CM preparations [61,62]. Subsequently, the scientific community apparently found it difficult to believe results pointing to the contrary (see [65,66] for review) and a considerable time span elapsed until it became accepted that all cyanobacteria known to date contain aa_3-type cytochrome *c* oxidase in both ICM and CM (separated membranes from >33 different strains and species biochemically tested so far [67–77]). At least three further remarkable facts have also prevented the acceptance of an independent, autonomous and self-sustained role of cyanobacterial respiration [76] (see [55] for the latest review): Most cyanobacteria are obligate phototrophs, unable to thrive efficiently in the dark at the expense of respiration on any organic substrate. The rates of cyanobacterial (endogenous) respiration are extraordinarily low (between 1% and 10% of maximum photosynthetic rates) and barely stimulated by "respiratory" substrates. And last, but not least, the only electron transport quinone that occurs in cyanobacteria in significant amounts is plastoquinone. And plastoquinone fits to oxygenic photosynthesis much better than to any type of respiration. After all, however, there is no doubt that in cyanobacteria both photosynthesis and respiration use plastoquinone as the predominant, if not only, electron transport quinone (see below).

3. A snapshot of the cyanobacterial respiratory chain

Instead of reiterating in detail the still somewhat controversial discussion on cyanobacterial RET [55], we will content ourselves with presenting a comprehensive, self-explanatory scheme of the membrane-bound electron transport (and other bioenergetically relevant) systems in Fig. 3A, and will only briefly highlight a few major peculiarities of cyanobacterial RET. First of all, as in most other phototrophic prokaryotes, the ICM contain a dual-function PET-RET system ([78]; see [45,65,66]). Yet, somewhat different from anoxyphototrophs, CM contain a pure-bred respiratory chain without photosynthetic reaction centres (Fig. 3A). Fig. 3B illustrates the (dark, aerobic) oxidation and reduction of horse heart Cyt *c* by one of the first preparations of isolated and separated cyanobacterial CM and ICM in our laboratory [67–77] showing thereby, at a glance, that also CM must contain a complete and functional respiratory chain. The distinct physical partition of the two membrane types (CM and ICM) in cyanobacteria (Fig. 3A) is more similar to a chloroplast (chlorophyll-free boundary membrane and thylakoid membranes without obvious anastomoses; see [79]) than to an anoxyphototrophic bacterium (multifarious invaginations of the CM forming various ICM structures [80], which latter, when isolated, are called *chromatophores*).

Cyanobacterial RET comprises, as any other biological RET system, the following five invariant components (from

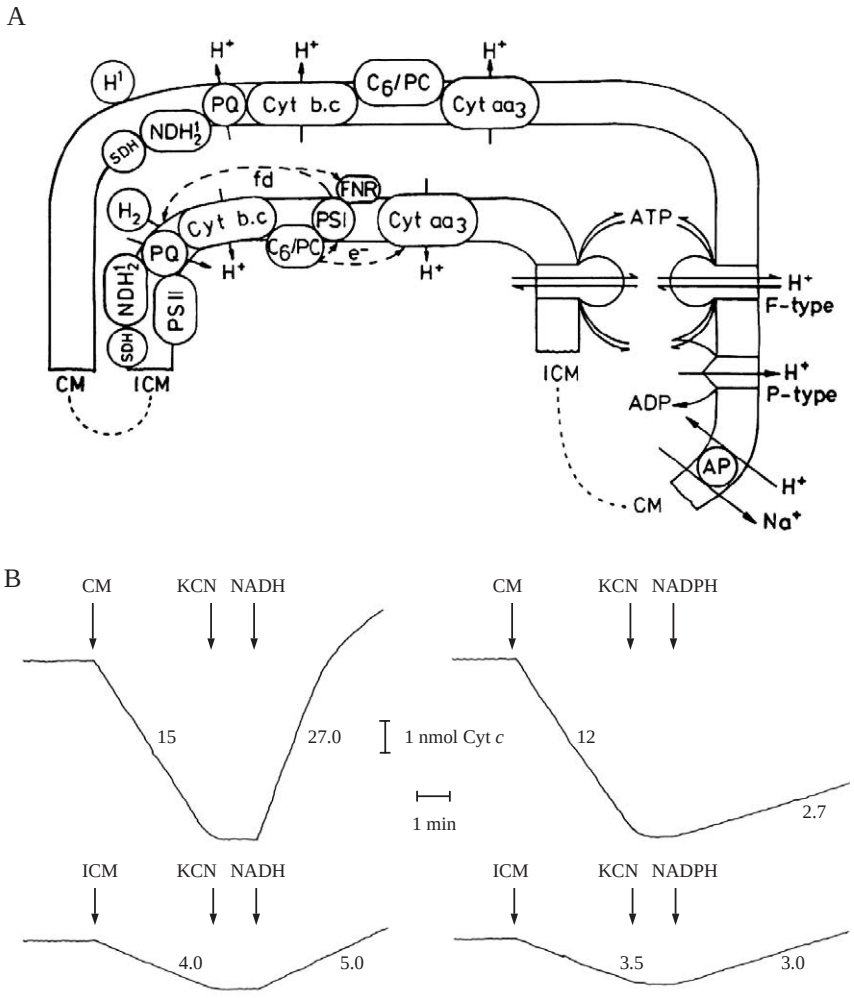


Fig. 3. (A) Scheme of bioenergetic membrane functions and sidedness in a model cyanobacterium. Particularly note the dual-function photosynthetic-respiratory chain (PET-RET) in the chlorophyll-containing ICM as contrasted to the pure-bred but otherwise identical respiratory chain (RET) in the chlorophyll-free CM. PSII, PSI, photosystem II and I; PQ, plastoquinone; fd, ferredoxin; FNR, fd-NADP$^+$ reductase; PC, plastocyanin (may be functionally replaced in some species by water-soluble Cyt c_6 in response to availability of Cu^{2+}); Cyt, cytochrome; NDH^1, NDH-1 enzyme, and NDH_2, NDH-2 enzyme (see text for explanation); H^1, bidirectional or respiratory hydrogenase; H_2, uptake or photosynthetic hydrogenase (see text for explanation); SDH, succinate dehydrogenase; H^+-pumping P-type ATPase (ATP hydrolase) and F-type ATPase (ATP synthase), and the Na^+/H^+ antiporter (AP) are also shown. Dashed lines between CM and ICM indicate hypothetical anastomoses between the two membrane systems. For in-depth discussion, see [55]. (B) Fundamental respiratory redox reactions (reduction and oxidation of exogenous horse heart Cyt *c*) as catalysed by the isolated and purified CM and ICM particle preparations from *A. nidulans* (*Synechococcus* sp. PCC 6301). Qualitatively identical results were obtained on CM and ICM from >33 different cyanobacteria [67–77]. These findings constituted first solid evidence that both cyanobacterial ICM and CM contain a complete respiratory chain from NAD(P)H to O_2. Oxidation of ferrocytochrome *c* (downward deflection) and reduction of ferricytochrome *c* at the expense of exogenous NAD(P)H (upward deflection) were measured by dual wavelength spectrophotometry at 550–540 nm. Figures adjacent to the traces give rates in nmol Cyt *c*/min per mg membrane protein as originally determined [70,71].

low (−) to high (+) potential; also see Fig. 3A): One or more dehydrogenases, an electron transport quinone (lipid-soluble mobile carrier), a Cyt *bc* complex, PC and/or Cyt c_6 (water-soluble mobile carrier), and a terminal respiratory oxidase (TRO) as the final electron acceptor ("electron sink"). For chemiosmotic energy conversion [12,13], it is obligatory that the membranes form a closed, osmotically autonomous compartment and that the same membranes also possess a reversible F_0F_1-ATPase or ATP synthase of appropriate orientation (see Fig. 3A) to catalyse the endergonic synthesis of ATP from ADP and P_i ("phosphorylation"). This enzyme, in functional terms, is the most strictly conserved biochemical device in the whole biosphere and certainly was present even before any sort of electron transport was "invented" [15–18]. The striking similarity of respiratory and photosynthetic electron transport chains with respect to both bioenergetic function and basic structural composition is a major proof of the conversion hypothesis (see above).

Significant chemical deviations from the common theme of electron transport components are found in the so-called *archaea* only [81,169,170]. In this context, the interesting note may be appropriate that up to the present day not a single *phototrophic* archaeon has been discovered and that even among Gram-positive eubacteria phototrophy is a rare

exception, whatever this striking correlation might mean in systematic terms. More prominent, yet still pace-keeping variations of the common *monophyletic* scheme (between chloroplasts and mitochondria, in particular) are the following: Photosystem II instead of dehydrogenases, plastoquinone instead of ubiquinone, a Cyt b_6f instead of a bc_1 complex, and Photosystem I instead of a TRO (see below). Fig. 3A nicely shows how the whole bioenergetic dichotomy somehow merges in a cyanobacterium which, therefore, might be rightly looked at as a free-living *chloromitochondrion* [45,46].

As to the low-potential end of RET, the physiological functions of both a respiratory ("reversible", see H^1 in Fig. 3A) and a photosynthetic ("unidirectional" or uptake) hydrogenase (see H_2 in Fig. 3A) had been described, obviously as an evolutionary relic, in the obligately phototrophic, unicellular, and non-nitrogen-fixing cyanobacterium *Anacystis nidulans* (*Synechococcus* sp. PCC6301) many years ago already [82–86]. These results were later confirmed by the tools of gene sequencing ("molecular biology") [87]. Generally, hydrogenases are extremely widespread and quite randomly distributed among all bacteria including cyanobacteria (for reviews, see [88–90]). In dinitrogen-fixing (cyano)bacteria, uptake hydrogenases are useful for the recycling of the 25% electrons inevitably going to H^+ instead of to N_2 [91] owing to some weird, but fundamental congenital 'defect' of the enzyme. In obligately aerobic hydrogen bacteria, uptake hydrogenases act as ultimate electron donors to the TRO of ordinary respiratory chains while in obligately anaerobic methanogens uptake hydrogenases reduce the alternative terminal electron acceptor CO_2 in a membrane-bound respiratory and chemiosmotic electron transport reaction giving rise for the production of CH_4 [81]. In many other anaerobic species, "reversible" hydrogenases help to prevent metabolic overreduction in the absence of suitable external oxidants ("hydrogen relief valve" [92]). It should be noted in this context that hydrogenases sensu stricto metabolize H_2 (either consuming or producing it) without hydrolysis of ATP while H_2 production by nitrogenase, which, in usual physiological conditions, mediates the hydrogen relief valve (see above [92]), needs, as for typical triple bond reductions (reduction of N_2, CN^-, HN_3, N_2O, C_2H_2) on an average 2 ATP/e^- transferred from the Fe- to the MoFe-protein. This net energy requirement (ATP hydrolysis) for thermodynamically exergonic reductions such as N_2 fixation (with H_2 or Fd) remains enigmatic: A primordial (in a reducing environment) energy-requiring hydrogenase might, in the course of evolution, have evolved into a nitrogenase as we know it today [93–95]. Note that both hydrogenase and nitrogenase are low-potential FeS enzymes operating at around $E^{o\prime}$=−420 mV and that both are severely inactivated or even damaged in the presence of free O_2, the latter usually even more than the former.

The occurrence of a "mitochondrial" energy-transducing, multisubunit NADH dehydrogenase in both CM and ICM of cyanobacteria as well as in chloroplasts (i.e. in *oxyphototrophs*) was first described by Steinmüller et al. [96,97]. Competent reviews on mitochondrial and bacterial energy-transducing (i.e. proton-pumping) NADH dehydrogenases, the so-called NDH-1 enzymes (NDH^1 in Fig. 3A), may be found in [98–102]. However, in all oxyphototrophs, 3 of the 14 NDH-1 subunits that form the minimal functioning complex I [102], viz. NuoE, F, and G, which make up the dehydrogenase module proper [97], are not coded for at all. Also, in contrast to previous claims, hydrogenase genes *hoxE*, *F*, and *U* do not substitute for *nuoE*, *F*, and *G* genes [103]. So, how can the more than well-established oxidation of NAD(P)H by cyanobacterial membranes be explained at all with an enzyme lacking the dehydrogenase module? For NADPH oxidation in cyanobacteria, soluble photosynthetic FNR once was invoked which, however, cannot be reconciled with solid experimental facts (see [55] for detailed discussion). Second, cyanobacteria synthesize two quite different types of NADH dehydrogenases: The classical multisubunit NDH-1 enzyme pumps protons, uses FMN and several FeS clusters as co-enzymes, oxidizes both deamino-NADH and NAD(P)H, and is strongly inhibited by rotenone or piericidin A (this enzyme is marked NDH^1 in Fig. 3A). The alternative NDH-2 enzyme (see NDH_2 in Fig 4A) usually consists of one subunit only, does not pump protons, utilizes FAD instead of FMN, and is devoid of FeS clusters. The (non-energy-transducing) NDH-2 enzyme does not oxidize NADPH [104] nor deamino-NADH while the cyanobacterial NDH-1 enzyme is assumed to oxidize both NADH and NADPH (as well as deamino-NADH) [115,117] most probably just *because* it is lacking the advanced 'mitochondrial' NADH dehydrogenase module. In several protists, NDH-2 functions as an energy-overflow valve removing excess reducing equivalents without at the same time giving rise for ATP synthesis [97].

Another potential confusion surrounding cyanobacterial NAD(P)H dehydrogenation is the following: At least two of the *ndh* (=*nuo*) genes are multicopy genes, each copy with a distinct primary structure, leading to six chemically (and possibly also physiologically) different proteins for subunit 4 and three different proteins for subunit 6 of the cyanobacterial NDH-1 complex. This way, in principle, each of the different holoenzymes could catalyse for a distinct physiological function e.g., in photosynthesis [97], respiration [108], and possibly even C_i transport [109] as has, indeed, been envisaged (see [55] for discussion). The

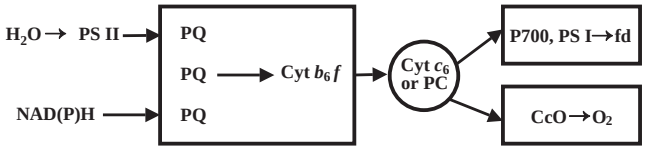


Fig. 4. The dual-function PET-RET assembly in cyanobacterial ICM. Note that, contrary to previous claims [152], either PC or Cyt c_6 is absolutely indispensable for integral electron transport in both photosynthesis and respiration [144,145].

preliminary kinetic characterization of NAD(P)H dehydrogenation by CM and ICM preparations of cyanobacteria has yielded the following three distinct K_M values: 5 and 25 μM toward NADH in both CM and ICM (to be attributed to NDH-1 and NDH-2, respectively), only one K_M toward NADPH in CM (due to the primordial NDH-1 enzyme of oxyphototrophs) but again two distinct K_Ms, of 5 and 2 μM, toward NADPH in ICM due to the bifunctional 'oxyphototrophic' NDH-1 and the photosynthetic FNR, respectively. These results were obtained on isolated and purified CM and ICM from *Synechococcus* 6301, *Synechocystis* 6803, *Anabaena* 7120, and *Nostoc* 8009 ('Mac'), they were strikingly uniform (i.e., each characteristic of a distinct and individual enzyme) and reproducible within 10–20% throughout [115,117]. Exclusive oxidation of NADPH by FNR as had previously been envisaged [116] could be ruled out by the observation of NADPH oxidation in CM preparations with which a monospecific antibody raised against cyanobacterial FNR did not react at all while it did react with ICM [55,115]. Succinate oxidation by the same membrane preparations, using either ambient O_2 or FeCy as oxidants, gave K_M values (toward succinate) of between 600 and 800 μM [117]. Further (including molecular) details of the dehydrogenation of NAD(P)H and succinate in (cyano)bacteria (see Fig. 3A) are discussed in [104–117].

A further remarkable peculiarity of cyanobacterial RET is the practically exclusive role of plastoquinone as the lipid-soluble mobile carrier in both CM and ICM (Fig. 3A). No ubiquinone or menaquinone has ever been detected in any cyanobacterium, thus all the more pin-pointing cyanobacteria as kind of free-living chloroplasts according to the well-known (classical) endosymbiont hypothesis [118]. And if the strikingly differential effects of plastoquinone and phylloquinone for the reconstitution of PET and RET in quinone depleted (pentane extracted) membranes [119,120] might explain the tremendously different rates of RET and PET (in the order of up to 1:100) in cyanobacterial CM and ICM, respectively, still remains to be seen. Similarly, also if the (almost) exclusive occurrence of PQ in cyanobacteria has prevented the expression of a functional quinol oxidase in cyanobacteria [121–125] is unknown (see below); at least, not a single plastoquinol oxidase has become known in any respiring cell so far.

The next specialty of cyanobacterial RET is an apparently chimaeric cytochrome *bc* complex. As has been mentioned already, b_6f complexes from the cyanobacterium *Mastigocladus laminosus* [51] (as well as from *Chlamydomonas rheinhardtii* chloroplasts [52]) have been structurally resolved in all details quite recently (whereby a hitherto overlooked Cyt *c* was discovered within the complex), and the occurrence of "split" b_6f complexes in chloroplasts and cyanobacteria (and in Gram-positive bacilli [126]) had been known for decades. However, it sprang a surprise when it was shown that the cyanobacterial b_6f complex occurs in both ICM and (chlorophyll-free!) CM [131,132] (Fig. 3A), that it is immunologically cross-reactive with monoclonal and strictly monospecific antibodies raised against Cyt c_1 from beef heart mitochondria and *Paracoccus denitrificans* [134], though cytochromes *f* and c_1 do not share sufficient amino acid homology [133], and that it is strongly inhibited by the classical complex III inhibitor antimycin *A*, which normally does not at all affect canonical b_6f complexes [108,134]. A similarly chimaeric nature has been suggested for the *bc* complexes from green sulfur bacteria [135] and heliobacteriaceae [136]. Extensive reviews on structure–function relationships of Cyt *bc* complexes are found in [127–130].

A last peculiarity–not only of cyanobacterial RET but of biological electron transport in general, be it PET or RET–is the "gap" between complex III (Cyt *bc* complex) and the terminal electron sink, viz. P700 of Photosystem I in PET or the TRO (complex IV) in RET. Apparently, this gap cannot be "filled" by the membrane itself, so to speak, but must be bridged by an extrinsic, water-soluble mobile carrier, either the blue copper protein PC in PET (an acidic protein in higher plant chloroplasts) or by Cyt *c* in RET (a strongly basic protein in mitochondria), respectively. Certain (respiring) bacteria, usually Gram positive bacteria and archaea, short-circuit RET between the quinone and complex IV by using a QOX instead of a CcO (see Fig. 3A); in doing so, they not only by-pass both complex III and the soluble mobile carrier but also renounce an important coupling site for ATP synthesis. Accordingly, also from this "economic" point of view, it seems logical to place CcO in evolution earlier than QOX (see below; also see [181]). In cyanobacteria, the gap is bridged by Cyt c_6 and/or PC (Fig. 4). Contrary to previous claims [152], at least one of the two redox proteins is absolutely indispensable for integral PET and RET [144,145] and in cyanobacteria both proteins, which can substitute for each other, are capable of reducing P700 of Photosystem I and the CcO [137–143], reaction rates primarily depending on IEPs of the redox proteins involved as shown by corresponding Brönsted plots [145–151]. Kinetic details of the electron transfer from *Synechocystis* PC and Cyt c_6 to the soluble membrane-extrinsic Cu_A domain of the TRO, using recombinant electron transport proteins of *Synechocystis* throughout, are being investigated in our laboratory using stopped-flow techniques [141–143].

4. The terminal oxidase(s) of cyanobacteria

In the respiratory sequence (Fig. 3A), we now come to the TRO (complex IV), to which the rest of this review will be devoted. The TRO is rightly designated the key enzyme of (aerobic) respiration. Its main task is reduction of O_2 to water in a concerted four-electron transfer without setting free dangerous ROS (see above; for general reviews, see [2,153,154]): $E^o(O_2/H_2O) = +820$ mV corresponding to a free energy (more strictly: *enthalpy*) gain of −478.6 kJ/mol

in the reaction O_2+2 H_2 ⇆2 H_2O, or −440 kJ/mol for O_2+2 NADH+2 H^+ ⇆2 H_2O+2 NAD^+, or −405 kJ/mol for O_2+4 H^++4 Cyt c^{2+} ⇆2 H_2O+4 Cyt c^{3+}, which is used for the electrogenic pumping of protons through the membrane-bound haem–copper oxidase [155] from the N- to the P-side of the excitable (energizable) membrane (inner mitochondrial membrane or bacterial CM, direction of H^+ movement outward of mitochondrion or cell; see Fig. 3A). *Mutatis mutandis*, at the expense of absorbed light energy, the same is brought about, via redox intermediates in the membrane, by the PET in chloroplast, cyanobacterial or other thylakoid membranes (ICM; see Fig. 3A), this time, however, the direction of H^+ movement is directed inward into the thylakoid lumen. The proton imbalance (=both electrical and concentration gradient) thus established across excitable membranes can relax (return to equilibrium) when the protons return through a likewise membrane-bound F_oF_1-ATPase (ATP synthase) from the P-side back to the N-side of the membrane thereby giving rise for the endergonic synthesis of ATP from ADP and P_i by the catalytic F_1 part of the enzyme on the N-side ("inside") of the membrane (see Fig. 3A; also [156] for detailed structure–function considerations of this oldest and absolutely ubiquitous bioenergetic enzyme in general, and [157] for a more quantitative treatment of cyanobacterial proton-pumping). In a biological overall sense, the TRO reaction (reducing O_2 to H_2O) thus

Table 1
Genes of haem–copper oxidases oxidases in nine cyanobacterial strains where the whole genome was sequenced

	Cytochrome *c* oxidase	Haem–copper quinol oxidase	Cytochrome *bd* quinol oxidase
Gloeobacter violaceus PCC7421	SU I, *ctaD* (glr0740) SU II, *ctaC* (glr0739)	–	SU I, *cydA* (gll1197) SU II, *cydB* (gll1196) [similar to SU II (gsr1544)]
	SU I, *ctaD* (gll2163) SU II, *ctaC* (gll2164) SU III, *ctaE* (gll2162)		
Nostoc (*Anabaena*) sp. PCC7120	SU I, *coxA* (alr0951) SU II, *coxB* (alr0950)	SU I, *coxA* (alr2732) SU II, *coxB* (alr2732)	SU I, *cydA* (all4024) SU II, *cydB* (all4023) [similar to SU I (asl0595)]
	SU III, *coxC* (alr0952)	SU III, coxC (alr2734)	
	SU I, *coxA* (alr2515) SU II, *coxB* (alr2514) SU III, *coxC* (alr2516)		
Prochlorococcus marinus MED4	SU I, *ctaD* (PMM0445) SU II, *ctaC* (PMM0446) SU III, *ctaE* (PMM0444) SU VIb? (PMM0814)	–	–
P. marinus MIT9313	SU I, *ctaD* (PMT1342) SU II, *ctaC* (PMT1341) SU III, *ctaE* (PMT1343) SU Va? (PMT1850)	–	–
P. marinus SS120	SU I, *cyoB* (Pro0441) SU II, *cyoA* (Pro0442) SU III, *cyoC* (Pro0440)	–	–
Synechococcus sp. WH8102	SU I, *ctaD* (SYNW1862) SU II, *ctaC* (SYNW1861) SU III, *ctaE* (SYNW1863)	SU I, *ctaD* (SYNW1529) SU II, *ctaC* (SYNW1528) SU III, *ctaE* (SYNW1530)	–
Synechocystis PCC6803	SU I, *ctaDI* (slr1137) SU II, *ctaCI* (slr1136) SU III, *ctaEI* (slr1138)	SU I, *ctaDII* (slr2082) SU II, *ctaC* (sll0813) SU III, *ctaEII* (slr2083)	SU I, *cydA* (slr1379) SU II, *cydB* (slr1380)
Thermosynechococcus elongatus BP-1	SU I, *ctaD* (tll2010) SU II, *ctaC* (tll2011) SU III, *ctaE* (tll2009)	–	SU I, *cydA* (tll1602) SU II, *cydB* (tll1601)
Trichodesmium erythraeum IMS101	SU I (gene 2511) SU II (gene 2510) SU III (gene 2512)	SU I (gene 5549) SU II (gene 5548) SU III (gene 5550)	–

The accession numbers are depicted in parentheses. SU, subunit.
Genes of haem–copper oxidases (both CcOs and QOXs) and of non-energy-transducing *bd*-type QOXs in nine cyanobacterial species whose entire genomes had been sequenced. (See beginning of abstract section for internet references). Accession numbers are given in brackets. Important note: in marked contrast to all the beautiful TRO genes that have been cloned and sequenced from cyanobacteria, the only TRO clearly identified so far as a functional protein in isolated and purified membranes from >33 cyanobacterial strains and species (using spectroscopy, immunoblotting and kinetic measurements) is the canonical aa_3-type CcO with a measured H^+/e^- ratio close to 1.0 in spheroplasts, vesicles, and proteoliposomes [63–77,206,216–220]. Thus far, apart from indirect genetic 'evidence', there is no single report from any research group around the world [125,222,223] disclosing the existence of any functional alternative TRO proteins in any cyanobacterium ever investigated. Yet, organisms ecologically as successful as the cyanobacteria, but nevertheless addicted and restricted to the utilization of light as the main energy source, should be assumed to find their way easily even without a multitude of different respiratory oxidases even though there might be (silent?) genes potentially coding for such teleologically and practically 'useless' enzymes.

formally reverses O_2 production from water, thereby closing the oxygen cycle (see Eq. (1)).

Returning now to the TRO of cyanobacteria, a few basic facts should be recalled: TROs can best be functionally classified as large superfamilies of haem–copper oxidases [153,154] and non-copper or *bd*-type oxidases [159,160]. A unifying property of the former is electrogenic proton translocation ("proton-pumping") through the membrane-bound oxidase [158]. The haem–non-copper oxidases, which often have an unusually high affinity toward O_2, are functioning in certain bacteria (e.g., N_2-fixing soil bacteria of the genus *Azotobacter*) as kind of energy overflow valve in conditions when efficient removal of O_2 is more important than ATP synthesis, i.e., in conditions known as "respiratory protection" [159–161], which also apply to N_2-fixing cyanobacteria, of course, particularly unicellular species [162].

In cyanobacteria, according to the genomic witness (see beginning of abstract above; also see the following, in particular Table 1) almost any type of terminal oxidase should be present, as if primordial (cyano)bacteria could have served themselves indiscriminately from an almost unlimited gene pool. Unfortunately, in several cyanobacteria, growth under a wide variety of conditions, including dinitrogen-fixing conditions (where applicable), did not give the least indication of the presence of any other functional terminal oxidase but the canonical aa_3-type CcO that had already previously been extensively characterized in >33 different species and strains of cyanobacteria without any indication of a functional alternative oxidase (see [55] for review). Preliminary reports as to the contrary, particularly those claiming the 'presence' of three different 'functional' oxidases, cannot be taken seriously as the conclusions rely on dubious whole-cell inhibitor studies while oxidase enzymes as such have never been identified and the many beautiful TRO genes thus could as well belong to the family of so-called silent genes, widely known from other living (particularly bacterial) cells, too. The expression of silent genes is either constitutively and permanently inhibited, or needs highly peculiar and extreme conditions for expression. Thus, what we are left with in cyanobacteria is the tedious (and moreover still lacking) analysis of innumerable genes that could potentially encode various terminal oxidases though it is well known that most of the cyanobacterial species in question are obligate phototrophs unable to sustain efficient growth and proliferation in darkness at the expense of respiration on any organic substrate. Why then, teleology asks, waste a lot of precious ATP for the synthesis of complicated transmembrane enzyme systems if these are unable after all to contribute anything to ecologically competitive survival? Doubtless, the predominant task of cyanobacterial respiration (note that fermentation is a still much less efficient energy conversion mechanism in cyanobacteria [163,164]) is the generation of maintenance energy during inevitable periods of darkness or when environmental stresses inhibit photosynthetic energy conversion [165]. It may be, however, that the rapidly developing and at present very fashionable DNA microchip array technique for the screening of gene expression, though extremely expensive, might yield useful results within the next few years already while, at present, nothing is known about a possibly differential expression of TRO genes in cyanobacteria. After all, the rest of this review will try to critically compare all possible terminal cyanobacterial haem–copper oxidases as derived from nine completely sequenced cyanobacterial genomes—together with terminal oxidase genes from four other cyanobacteria, and from *P. denitrificans*, *Rhodobacter sphaeroides*, *Escherichia coli*, *Thermus thermophilus*, and *Bos taurus* whose high-resolution 3D structures have been resolved (see below).

All haem–copper oxidases, in contrast to "constitutively uncoupled", non-energy-transducing *bd*-type oxidases (see

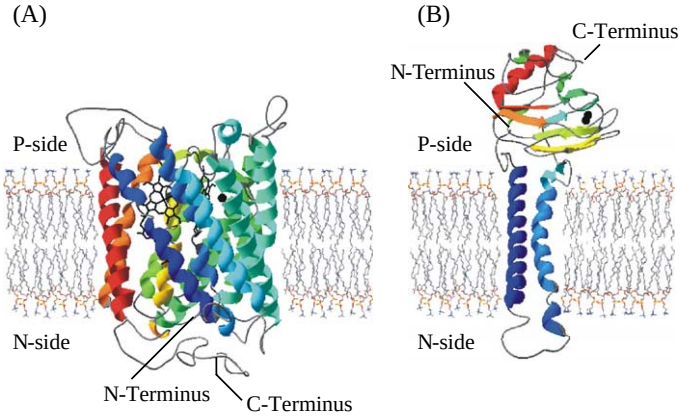


Fig. 5. Membrane-topographic modeling of subunits I (A) and II (B) of the CcO in *Synechocystis* sp. PCC6803 as deduced from the genes first cloned and sequenced in the late 1980s and subjected to Kyte–Doolittle evaluation [191–194]. Model building was based on the crystal structure of CcO from *P. denitrificans* [187] by using SWISS-Model and the SWISS-PdbViewer (see www.expasy.ch/spdbv/). Copper ions and haems *a* and a_3 are depicted in black. P-side is periplasmic in case of CM but intrathylakoid (lumenal) in case of ICM; N-side is cytosolic with both CM and ICM. For subunit III [192], the software currently available refused any model building as it was done for subunits I and II indicating major structural differences between cyanobacterial CcO subunit III and most other subunits III whatever this might mean in detail.

above), pump protons electrogenically across the membrane though in certain cases, e.g., the aberrant ba_3-type CcO from *T. thermophilus* [197–199] (a type *B* oxidase sensu Pereira et al. [179]) H^+/e^- stoichiometries may be lower than 1.0 (Fig. 6A and [199]). Quite generally, their subunits I may combine with different haem groups (*a*, *b*, *o*, and possibly even *d* [124]) as implicated by the term "promiscuity of haem groups" [166–169]. The same phenomenon has also been characterized in cyanobacterial CcOs in response to ambient oxygen concentrations [121–124]. Recalling the fact that, concerning haems *o* and *a*, for example, the transformation of the respective methyl group into the corresponding formyl group (e.g., 7-methyl chl-*a* to 7-formyl chl-*b* [171], analogous to 8-methyl haem-*o* to 8-formyl haem *a*) is catalysed by an oxygenase enzyme, and furthermore considering oxygenases' affinity toward O_2 generally being only 10^{-2} that of oxidases [172], an influence of the O_2 partial

(A)HELIX VI of subunit I:

The paradigms:

B.tau.	HLFWFFGHPEVY	E-242
R.sph.	HILWFFGHPEVY	E-286
P.den.	HILWFFGHPEVY	E-278

Cyanobacteria:

S.6803.1	HLFWFYSHPAVY	aa_3
S.6803.2	HLFWFYSHPAVY	bo_3?
S.6803.3	HLFWFYSHPAVY	*bd?*
Sc.vulc.	HLFWFYSHPAVY	aa_3
A.nid.	HLFWFYSHPAVY	aa_3
N.7120.1	HYFWFYSHPAVY	aa_3
N.7120.2	HLFWFYSHPAVY	?
N.7120.3	HMFWFYSHPAVY	?
N.7120.1	------------	aa_3?
N.punc.1	HLFWFYSHPAVY	aa_3
N.punc.2	------------	?
N.punc.3	HYFWFYSHPAVY	?
N.punc.4	HLFWFYSHPAVY	?
N.punc.5	HMFWFYSHPAVY	?
Sc.WH8.1	HFFWFYSHPAVY	?
Sc.WH8.2	HLFWFYSHPAVY	aa_3
Ab.7937.1	HLFWFYSHPAVY	aa_3
Ab.7937.2	HLFWFYSHPAVY	?
Pro.MIT	HLFWFYSHPAVY	aa_3
Pro.ME	HLFWFYSHPAVY	aa_3

Others (including Archaea*):

E.coli.Q	NLIWAWGHPEVY	bo_3 (*A1*-type)	normal
S.acid.1*	QLFWFFGHPEVY	ba_3 (*A1*-type)	normal
S.acid.2*	ILFWFYGHPVVY	aa_3 (*A2*-type)	(+)
T.therm.	TLFWWTGHPIVY	ba_3 (*B*-type)	H/e=0.4
Ac.ambi.*	IAFWIFGHAVVY	aa_3 (*B*-type	(+)
Na.phar.*	TLFWYFGHAVVY	ba_3 (*B*-type)	(+)
R.mar.	HFFWFYSHPAVY	caa_3 (*A2*-type)	(+)
Br.jap.	MFQWWYGHNAVG	cbb_3 (*C*-type)	(+)
H.pylori	LIQWWWGHNAVA	cbb_3 (*C*-type)	(+)

P.den. Mutants[165]:

single	HILWFFGHPAVY	1.0% ET act.	no ptl
double	HILWFFSHPAVY	0.1% ET act.	no ptl
triple	HILWFYSHPAVY	10% ET act.	H/e=0.7

(B)Loop 2/3 between helices II and III of subunit I, the $N(X)_{10}D(X)_6N$-motif of the "D-channel"(D128):

B.tau.		IGGFGN---WLVPLMIGAPD----MAFPRMNNMSFWLLP
R.sph.		FGGFGN---YFMPLHIGAPD----MAFPRMNNLSYWLYV
P.den.		FGGFGN---YFMPLHIGAPD----MAFPRLNNLSYWMYV

Cyanobacteria:

S.6803.1		VG-LAN---YLIPLMIGARD----VAFPVLNAIAFWLMP
S.6803.2		AA-FAN---YLIPLMVGTED----MAFPRLNAVAFWLTP
S.6803.3		AA-FAN---YLIPLMVGTED----MAFPRLNAVAFWLTP
S.vulc.		GG-FGN---YLVPLMIGARD----MAFPRLNALAFWLNP
A.nid.		AG-FGN---YLIPFYVGARD----MAFPKLNALAFWLIP
N.7120.1		VG-LAN---YLVPLMIGARD----MAFPRLNAAAFWMVP
N.7120.2		IGGFGN---YLIPLMIGARD----MAFPKLNAIAFWLNP
N.7120.3		AG-FAN---YLIPLMIGARD----MAFPRLNAVAFWMIP
N.punc.1		IGGFGN---YLVPLMVGARD----MAFPKLNAIAFWLNP
N.punc.2		----------
N.punc.3		----------
N.punc.4		AG-LSN---YLVPLMIGARD----MAFPLLNAISFWILP
N.punc.5		AG-FAN---FLIPLMIGAKD----MAFPRLNAVAFWMIP
S.WH8.1		NG-FNN---LLIPTMIGAPD----MAFPKLNAAAFWLVP
S.WH8.2		NGAFGN---YLIPFYVGARD----MAFPRLNAVAFWLIP
Ab.7937.1		IGGFGN---YLIPLMIGARD----MAFPKLNAIAFWLNP
Ab.7937.2		AG-FAN---YLIPLMIGARD----MAFPRLNAVAFWMIP
Pro.MIT		NGAFGN---YLIPFYVGARD----MAFPRLNAVAFWMIP
Pro.MED		NGAFGN---YLIPFYVGARD----MAFPRLNAVAFWLIP

Others (including Archaea*):

E.coli.Q	bo_3	-IGLMN---LVVPLQIGARD----VAFPFLNNLSFWFTVV
T.therm.	ba_3	QAIMV----YLPARELNMR------PNMGLMWLSWWMAFI
Ac.ambi.*	aa_3	EFALFI----
YFTIKLLNLQPRAKWLLNTAFIAINISMMF		
Na.phar.*	ba_3	LVGIFTWAVTTSLDRSLGNIR----
FTQTWYGLMTLGTVL		
S.acid.1*	ba_3	-TGFAN---YLVPRMIGAHD----LYWPKINALSFWMLVP
S.acid.2*	aa_3	AAAVIGFS-LYKSKLSI---------IHTKQMAIFFWLS-
R.mar.	caa_3	PAVLGN---FALPIMIGARD----VAFPRLNLASWYIFWL
Br.jap.	cbb_3	IATSF----YVVQKSCRVR------LAGDLAPWFVVVGYN
H.pylori	cbb_3	WASWY----YIGQRVLKITYHóQHPFLKIVGLLHFWLWII

Fig. 6. Alignment of amino acid sequences in orthodox and modified D-pathways in helix VI (A) and on the 2/3 loop between helices II and III (B) of subunits I of proton-pumping CcOs of (cyano)bacteria featuring the canonical **FG**HP**E**VY/**YS**HP**X**VY and **N**$(X)_{10}$**D**$(X)_6$**N** motifs, respectively. For comparison, also the aberrant yet structurally fully resolved CcO from *T. thermophilus* [197,198], which exhibits a low H^+/e^- efficiency only [199], is included. In addition to the nine "completely sequenced" cyanobacterial species, the table also contains TROs from *Nostoc punctiforme* (www.jgi.doe.gov/), *Anabaena* sp. PCC7937 [179], *Synechococcus vulcanus* [195], and *A. nidulans* (*Synechococcus* sp. PCC6301) [G.A. Peschek and the late Dr. Manuel Simon 1996, Centre for Applied Genetics, Agricultural University of Vienna, Austria, unpublished]. Note that *Anabaena* PCC7120 is synonymous to *Nostoc* PCC7120, and that, on a functional basis, in cyanobacteria, proton-pumping (with a H^+/e^- stoicheiometry of close to 1.0) has been verified so far only with carefully prepared, absolutely intact sphaeroplasts of *A. nidulans* oxidizing exogenous Cyt's *c* ([216–219], as had initially also been done with *P. denitrificans*, however [220]), and with notoriously tiny quantities of isolated and purified liposomal CcOs from *Anacystis* and *Synechocystis* [206]. The same preparations also gave clear-cut evidence for adenylate regulation of the rates of Cyt *c* oxidation and H^+ extrusion by vesicle- or liposome-entrapped MgADP (up-regulation) and MgATP (down-regulation) similar to previous findings with beef mitochondrial CcO [221]. Yet, the latter results in particular, obtained on a three-subunit prokaryotic CcO, are far from understood even though they clearly would make much sense for the regulation of cellular energy metabolism [221]. Abbreviations: *A*,*B*,*C*-classification according to [179]; etf act., electron transfer activity; ptl, proton translocation; (+), qualitatively verified. Abbreviations of additional organisms: A, *Anacystis* (*Synechococcus* sp. PCC6301); Ab, *Anabaena*; Sy, *Synechocystis*; N, *Nostoc*; Sc, *Synechococcus*; Pro, *Prochlorococcus*; S, *Sulfolobus*; Th, *Thermus*; Ac, *Acidianus* ; Na, *Natronobacterium*; Rb, *Rhodobacter*; Br, *Bradyrhizobium*; H, *Helicobacter*.

pressure on the differential expression of haem groups *o* and *a* is well conceivable, and still more 'reducing conditions' might even favour the synthesis of still more reduced haem *d*.

Second, the immediate electron donors to haem–copper oxidases may be either water-soluble *c*-type Cyt's (CcOs) or lipid-soluble quinols (QOXs). Following pace-making previous developments [173–176], the binuclear Cu_A centre on the *P* side (extracellular or intrathylakoid side) of subunit II (see the schemes in Figs. 3A and 5B) has been identified as the initial site of entrance of electrons from Cyt *c* while a series of conserved polar amino acids within the membrane core have been suggested as the quinol-binding site on subunit I of the QOX from *E. coli* [177]. Note that both types of haem–copper oxidases, irrespective of electron donors or haem groups employed, are canonical proton pumps: Their subunits I contain at least the two best-known H^+ channels, the D-channel for *pumped* protons and the K-channel for *chemical* protons, even if according to most recent results either can be used for both [178–180]. Interestingly, and a bit confusingly, both types of haem–copper oxidases are almost randomly distributed over the kingdoms of eubacteria and archaea, and slightly modified but equally efficient "channels" have become known, particularly through the in-depth study of Teixeira and associates [179,180]. Given the geochemical unlikeliness of the "respiration–early" hypothesis [28] (see above), it is hoped that the following discussion, which presents the first comprehensive overview on cyanobacterial terminal oxidases, will help to corroborate and streamline the necessarily protagonistic role of cyanobacteria as the homes of the first respiratory O_2 reductase [181].

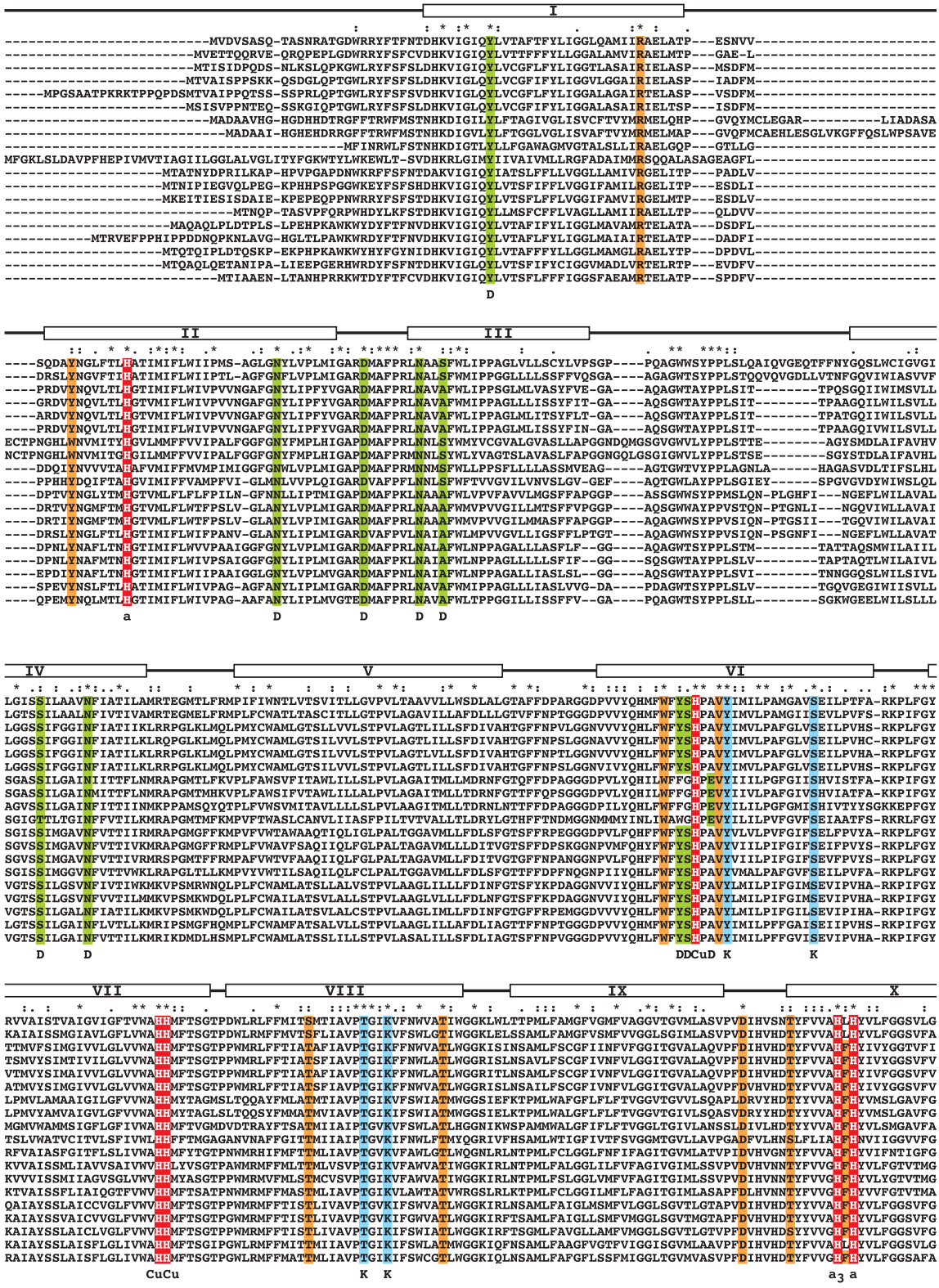
On the grounds of amino acid sequence similarities and the specific electron donor, viz. Cyt *c* or (*plasto*-)quinol, respectively, cyanobacterial haem–copper cytochrome *c*/quinol oxidases are here, for clarity, separated into three groups, true CcOs, putative haem–copper QOXs, and putative Cyt *bd* QOXs, as depicted in Table 1. Molecular details and sequence accession numbers of the two Cyt *bd*-type QOXs from *E. coli* can be found in [182–184]. The *bd* oxidases do not contain copper but usually two haems *b* and one haem *d*, do not act as electrogenic proton pumps, and do not show any sequence similarity with either of the two groups of haem–copper oxidases [182–184]. They are therefore excluded from the present discussion since, as for cyanobacterial quinol oxidases in general, there is nothing known up to the present day about functional enzyme proteins bearing the alleged functions. Be it emphasized that any H^+ transfer from the solvent phase outside the membrane to an intramembrane reactive centre, in which protons are taking part, must necessarily be conducted through an intraprotein H^+-conducting network irrespective of the specific H^+-consuming or H^+-releasing reaction. This applies a fortiori also to the four scalar or chemical protons that are consumed in the reduction of O_2 to 2 H_2O. Such H^+ conductivity does not necessarily need protonable residues on the protein backbone. Flexible water chains, either associated with polar residues or simply gathered in even hydrophobic microcavities of the protein scaffold, can assure H^+ transport along an even mostly hydrophobic, at least nonprotonable protein scaffold. The highly unusual physicochemical properties of the water molecule and its peculiar mechanism of electrical conductivity (the Grotthuß mechanism first put forward by the German physicochemist T.(C.J.) Grotthuß in 1805) deserve special attention in this respect [185,186].

4.1. Subunit I

All haem–copper oxidases possess a strikingly well-conserved subunit I that binds the co-factors of the catalytic centres, the haem–copper binuclear site, and the low-spin haem. Until now, five high-resolution 3D structures of haem–copper oxidases are known including the three orthodox CcOs from *P. denitrificans* [187], *R. sphaeroides* [188] and *B. taurus* mitochondria [189,190], the aberrant CcO from *T. thermophilus* [198], and one haem–copper bo_3-type quinol oxidase viz. from *E. coli* [177]. Because of the known structures of these five haem–copper oxidases, they are included, for comparative purposes, in our discussion.

Amino acid residues coordinating the three metal centres are strictly conserved in all haem–copper oxidases: HisI-94, HisI-276, HisI-325, HisI-326, HisI-411, and HisI-413, using *Paracoccus* numbering for all amino acid residues (see highlighted histidines in Fig. 7). In prokaryotic haem–copper oxidases, various different haem groups can take over the roles of both low-spin haem *a* and high-spin haem a_3 of beef mitochondrial CcO (promiscuity of haem groups, see before), but the type of haem shows no correlation to the type of electron donor used for reduction of the metal centres nor does it imply any major amino acid similarities. All subunits I of haem–copper oxidases have at least 12 transmembrane helices but some have additional hydrophobic patches. For example, the QOX from *E. coli* has one additional transmembrane helix at the N-terminus and two at the C-terminus. In all the cyanobacterial subunit I sequences analysed in this paper no extra hydrophobic regions could be detected, so there should be no more than the 12 standard transmembrane helices present as had already been found at the time of the first cloning and sequencing of a cyanobacterial CcO, which had still to be done "by hand" at that time [191–195]. Fig. 5 shows a scheme of the membrane topography of subunits I and II of the typical cyanobacterial CcO (comprising at least three subunits in toto [191–195]). All cyanobacterial subunits I have a short loop between helices I and II, similar to many other subunits I [179] but much shorter than that of *P. denitrificans* or *R. sphaeroides*. There are no extended loops between the transmembrane helices comparable to other bacterial haem–copper oxidases. Thus, the 12 transmembrane helices of cyanobacterial subunits I seem to form an unusually

compact confinement (scaffold) for the redox centres. The C-terminal endings of cyanobacterial subunits I are normally about 10–20 amino acid residues longer than that of *P. denitrificans* or *R. sphaeroides*. Only CcO subunits I of *Trichodesmium erythraeum* and *Nostoc* PCC7120 have longer extensions containing many polar amino acids without any longer hydrophobic segment. In addition to the six histidine ligands of metal centres, several other



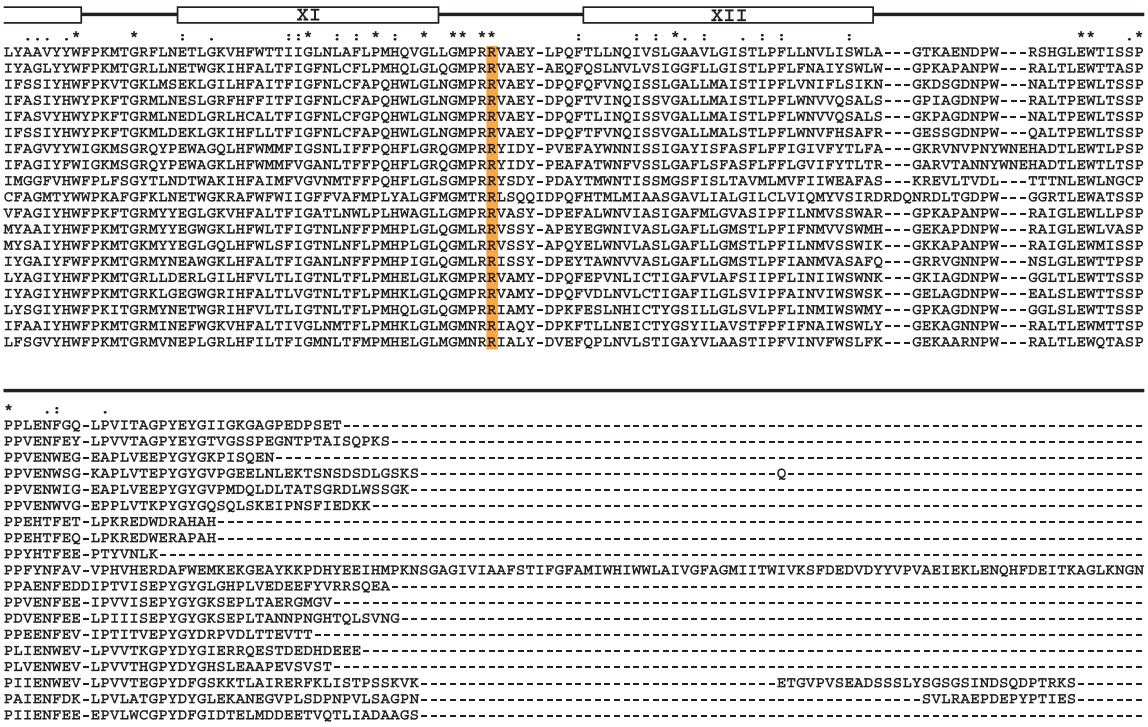


Fig. 7. Amino acid sequence alignment of subunits I of cyanobacterial haem–copper oxidases and four subunits I of oxidases whose 3D structure is known (gene assignments and accession numbers in brackets). The six invariant histidine ligands to low- and high-spin haems and to Cu_B are underlaid in red and marked by *a*, a_3, and Cu, respectively. Conserved amino acid residues are highlighted in orange. Additionally, amino acid residues of putative proton channels are labeled by D (D-channel; highlighted in green) and K (K-channel; highlighted in blue). Transmembrane helices of the *P. denitrificans* cytochrome *c* oxidase are indicated by rectangles on top of the alignment and marked by Roman numerals. Abbreviations: G.vio.1, *Gloeobacter violaceus* PCC7421 cytochrome *c* or quinol oxidase (glr0740; note the Q/E exchange); G.vio.2, *G. violaceus* PCC7421 cytochrome *c* oxidase (gll2163); P.MED4, *Prochlorococcus marinus* MED4 cytochrome *c* oxidase (PMM0445); P.9319, *P. marinus* MIT9313 cytochrome *c* oxidase (PMT1342); S.8102.1, *Synechococcus* sp. WH8102 cytochrome *c* oxidase (SYNW1862); PSS120, *P. marinus* SS120 cytochrome *c* oxidase (Pro0441); P.den., *P. denitrificans* cytochrome *c* oxidase (P98002); R.sph., *R. sphaeroides* cytochrome *c* oxidase (P33517); B.tau., *B. taurus* cytochrome *c* oxidase (P00396); E.coliQ, *E. coli* quinol oxidase (NP_414965.1); S.8102.2, *S.* sp. WH8102 quinol oxidase (SYNW1529); N.7120.3, *Nostoc* (*Anabaena*) sp. PCC7120 quinol oxidase (alr2732); T.ery.2, *T. erythraeum* IMS101 quinol oxidase (gene 5549); S.6803.2, *Synechocystis* PCC6803 quinol oxidase (slr2082); T.elo., *Thermosynechococcus elongatus* BP-1 cytochrome *c* oxidase (tll2010); N.7120.2, *Nostoc* (*Anabaena*) sp. PCC7120 cytochrome *c* oxidase (alr2515); T.ery.1, *T. erythraeum* IMS101 cytochrome *c* oxidase (gene 2511); N.7120.1, *Nostoc* (*Anabaena*) sp. PCC7120 cytochrome *c* oxidase (alr0951); S.6803.1, *Synechocystis* PCC6803 cytochrome *c* oxidase (slr1137). The multiple sequence alignment was produced with program T-COFFEE version 1.37 [215]. For abbreviations of the source organisms, also see Table 1.

amino acids are strongly conserved in subunits I of cyanobacteria as well as in other bacteria (see [179]). One is a valine of helix VI (ValI-279), which appears to be part of the oxygen diffusion channel [196]. A second conserved residue on helix VI is a tryptophan (TrpI-272), which interacts with one of the copper-coordinating histidines. An arginine (ArgI-474) in the loop between helices XI and XII hydrogen bonds to the low-spin haem *a* and is necessary for proton exit [180] and a second arginine (ArgI-54) on helix I likewise is hydrogen-bonded to this haem. In helix II, an aromatic residue forms a hydrogen bond to the haem. In *P. denitrificans* and *R. sphaeroides*, this is a tryptophan (TrpI-87), whereas in *B. taurus* and all cyanobacterial subunits I, this is a tyrosine. In helix VIII, a threonine (ThrI-344) or a serine forms a hydrogen bond to another copper-coordinating histidine. Only the two *Gloeobacter* subunits I contain a serine; all other cyanobacteria have a threonine at this place. Between helices IX and X, all cyanobacteria possess an aspartate (AspI-399), which hydrogen bonds to the high-spin haem, but other bacteria have an asparagine at this position. A phenylalanine (PheI-412) may be involved in electron transfer between the two haems, yet it is replaced by a leucine in both *Gloeobacter* subunits I and in *Nostoc* PCC7120 CcO subunit I. The C-terminus of helix VIII (ThrI-361) and the N-terminus of helix X (ThrI-405) contain a conserved threonine in most cyanobacterial subunits I except the *Trichodesmium* CcO subunit I, which has a serine at the N-terminus of helix X.

The five high-resolution 3D structures of the subunits I of haem–copper oxidases exhibit a core of 12 transmembrane helices that form three arcs within the membrane [178–180]. Each arc is shaped by four transmembrane helices and together with the last segment of the previous arc pore-like arrangements are formed. The first pore is filled with aromatic residues, the second holds the binuclear centre, and the third one the low-spin haem. Additionally, the first and

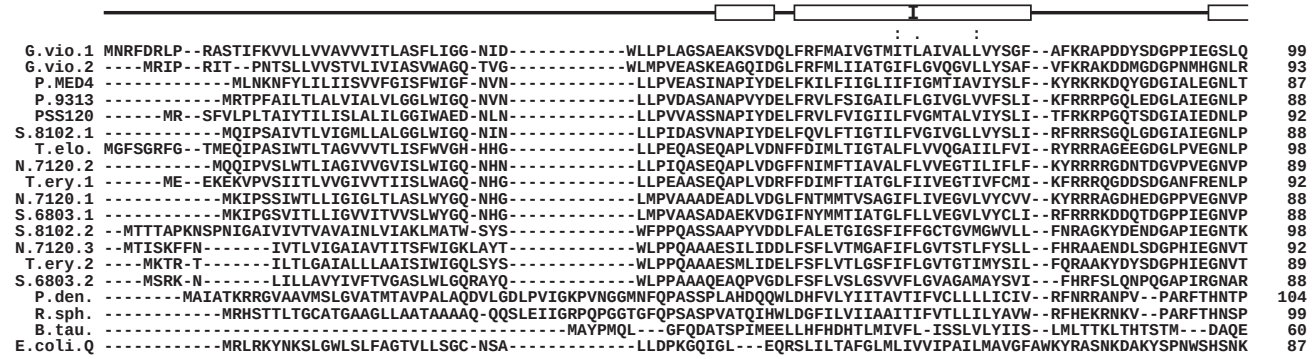
second pores form two uptake pathways for protons from the N-side, viz. the so-called D- and K-channels. The D-channel comprises, on helix VI (Fig. 7A) AspI-124 (D), AsnI-199, AsnI-113, AsnI-131, TyrI-35, and SerI-193 at the lower part of the first pore and the upper part of the second pore (see [178–180] for a more penetrating discussion of H^+ channels). The second part of the D-channel is located on the 2/3-loop between helices II and III of subunit I (Fig. 6B). Its primary structure is conserved in all cyanobacterial subunits I. This part of the D-channel forms a hydrophilic environment but afterwards hydrophobic residues dominate. Thus, water molecules must conduct the protons to the binuclear centre. A serine (SerI-134) as part of the D-channel is conserved in helix III in many bacteria but is replaced by an alanine in most cyanobacteria; only the two *Gloeobacter* subunits I also contain a serine. The K-channel comprises LysI-354 (K), ThrI-351, SerI-291, and ThrI-280 at the second pore, which leads directly to the binuclear centre [174]. Also, HisI-276 (ligand to Cu_B) and Tyr-280, which may form a covalent bond [197], are conserved in all cyanobacterial subunits I. The covalent bond between HisI-276 and TyrI-280, first identified in the crystal structure of *T. thermophilus* CcO [198] but likely to be present also in other–including cyanobacterial–CcOs, would make much sense in view of the possible formation of a transient tyrosyl radical during catalysis [198].

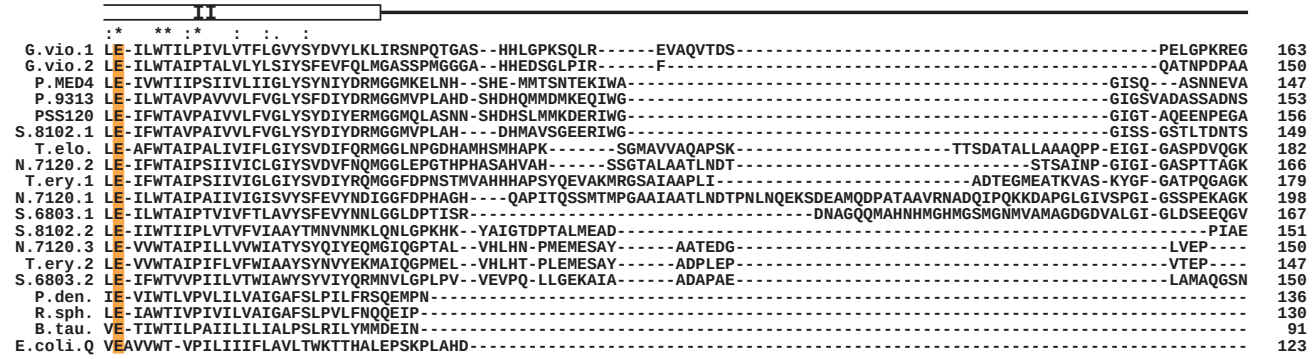
According to a novel classification of haem–copper oxidases [179], the latter can be divided into three families of oxygen reductases on the basis of sequence similarities of the core subunits I and II. Enzymes possessing the D- and K-channels (Fig. 7) are included in the type *A* haem–copper oxidases. Within this family, some of the proteins contain a conserved glutamate (GluI-278) on helix VI in the sequence motif -XGHP*E*V-, which seems to be decisive for proton transport; these oxidases are grouped as subfamily A_1 (E-containing oxidases). Other type *A* oxidases do not contain this glutamate but, instead, have a tyrosine and a consecutive serine on helix VI in the sequence motif -*YS*HP*X*V-. It was proposed that the tyrosine and serine, sterically occupying a position equivalent to GluI-278, might functionally substitute for the glutamate in proton transport [179,200]. Corresponding haem–copper oxidases were classified as subfamily A_2 (YS-containing oxidases). *All* cyanobacterial haem–copper oxidases are members of the YS-containing subfamily; they possess an alanine instead of the glutamate (Figs. 6A and 7).

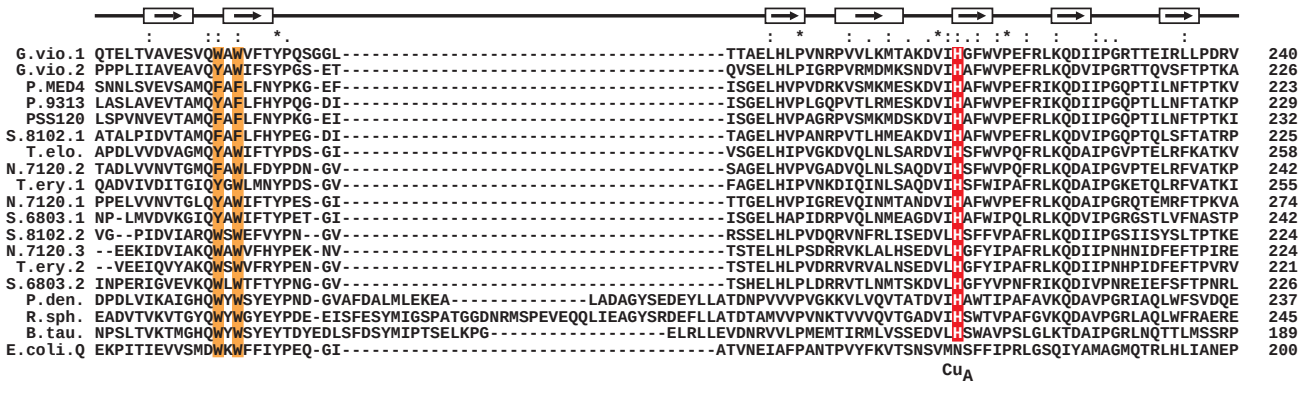
A second family of haem–copper oxidases are called *B*-type oxidases; they do not contain the conserved residues forming the D- and K-channels, but homologous channels may be present since H^+ pumping is experimentally verified (Fig. 6A). The third subfamily of haem–copper oxidases are the *C*-type oxidases; here, cbb_3 oxidases such as the well-characterized QOX from *Rhodobacter capsulatus* [201] are combined. Type *C* oxidases have no D-channel, and the alternative K-channel is only partly conserved ([179,180]; see Fig. 7). Nevertheless, all of them are capable of proton pumping, though perhaps at reduced efficiency [180,199]. Cyanobacteria do not encode type *B* or type *C* haem–copper oxidases.

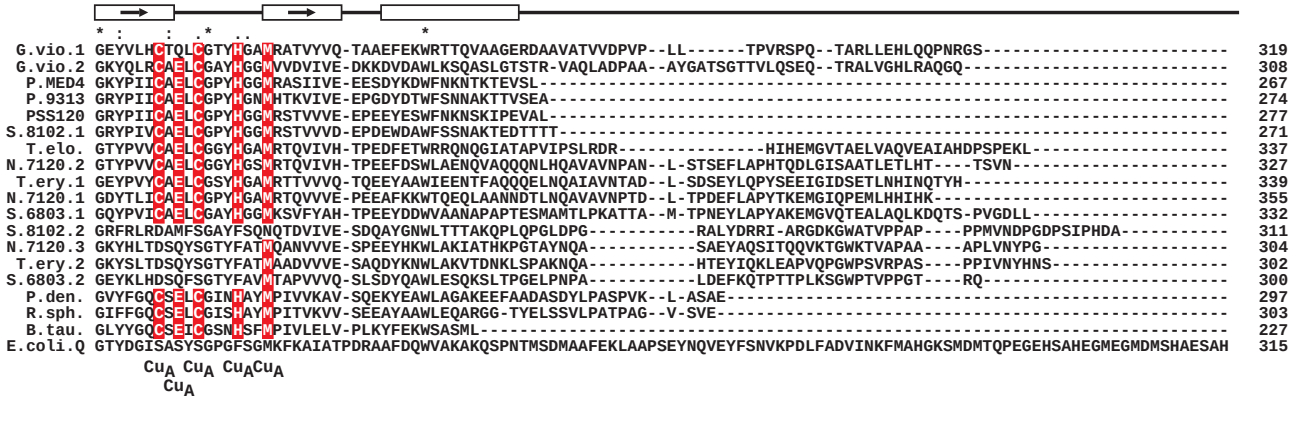
Putative quinol oxidases of cyanobacteria do not show the conserved polar residues at the end of helix I and the beginning of helix II, which *may* form the quinol-binding site of the QOX from *E. coli* [177]. *E. coli* bo_3-type QOX contains Arg-71 *plus* Asp-75 and His-98 *plus* Gln-101 (*E. coli* numbering), respectively, as polar amino acids in helices I and II, but in cyanobacterial haem–copper oxidases no such polar residues can be found in this region (Fig. 7). Thus, from the cyanobacterial sequences, no obvious electron transfer from (plasto)quinol to the low-spin haem can be deduced in those haem–copper oxidases where the corresponding subunits II do not possess a copper-binding site (see Fig. 8). Because of the absence of the Cu_A-site in subunits II, it was assumed that Cyt *c* cannot be the electron donor and the corresponding haem–copper oxidases were, perhaps prematurely, classified as QOXs [125] (also see Table 1). However, either cyanobacterial "QOXs" exhibit a novel quinol-binding site (but note that, from the viewpoint of an organic or physical chemist, plastoquinone is nevertheless very, very similar to ubiquinone—e.g., both are prenyl benzoquinones of similar redox potential), or they use an electron donor entirely different from all other haem–copper oxidases, or, most likely, they cannot at all form a functional QOX enzyme [121–125]. Yet, except cytochrome c_6, plastocyanin, and (possibly) plastoquinol, no other realistic candidates for direct electron transfer to the cyanobacterial TRO have been identified so far; the rather low concentrations of polar naphthoquinone and phyllo-

Fig. 8. Amino acid sequence alignment of subunits II of cyanobacterial haem–copper oxidases and four subunits II of oxidases whose 3D structure is known (gene assignments and accession numbers in brackets). Ligands to Cu_A underlaid in red and marked by Cu_A. Conserved amino acid residues are highlighted in orange. Transmembrane helices, α-helices, and β-strands of the *P. denitrificans* cytochrome *c* oxidase are indicated by boxed Roman numerals, rectangles and boxed arrows, respectively, on top of the alignment. Abbreviations: G.vio.1, *G. violaceus* PCC7421 cytochrome *c* or quinol oxidase (glr0739); G.vio.2, *G. violaceus* PCC7421 cytochrome *c* oxidase (gll2164); P.MED4, *P. marinus* MED4 cytochrome-*c* oxidase (PMM0446); P.9313, *P. marinus* MIT9313 cytochrome *c* oxidase (PMT1341); *P. marinus* SS120 cytochrome *c* oxidase (Pro0442); S.8192.1, *Synechococcus* sp. WH9102 cytochrome *c* oxidase (SYNW1861); T.elo., *T. elongatus* BP-1 cytochrome *c* oxidase (tll2011); N.7120.2, *Nostoc* (*Anabaena*) sp. PCC7120 cytochrome *c* oxidase (alr2514); T.ery.1, *Trichodesmium erythraeum* IMS101 cytochrome *c* oxidase (gene 2510); N.7120.1, *Nostoc* (*Anabaena*) sp. PCC7120 cytochrome *c* oxidase (alr0950); S.6803.1, *Synechocystis* PCC6803 cytochrome *c* oxidase (slr1136); S.8102.2, *Synechococcus* sp. WH8102 quinol oxidase (SYNW1528); N.7120.3, *Nostoc* (*Anabaena*) sp. PCC7120 quinol oxidase (alr2731); T.ery.2, *T. erythraeum* IMS101 quinol oxidase (gene 5548); S.6803.2, *Synechocystis* PCC6803 quinol oxidase (sll0813); P.den., *P. denitrificans* cytochrome-*c* oxidase (1311262A); R.sph., *R. sphaeroides* cytochrome *c* oxidase (Q03736); B.tau., *B. taurus* cytochrome *c* oxidase (OBBO2); E.coli.Q, *E. coli* quinol oxidase (NP_414966.1). The alignment was produced as in Fig. 7.






Cu_A


Cu_A Cu_A Cu_ACu_A
Cu_A

quinone (see above) are unlikely to play a role in physiological electron transport reactions. Thus, too much has still to be learned about the terminal segment of cyanobacterial RET before a firm conclusion can be drawn.

4.2. Subunit II

In addition to subunit I, cyanobacterial haem–copper oxidases always contain at least one other subunit, namely subunit II (Table 1; Fig. 8). Sequences of cyanobacterial subunits II include a putative N-terminal signal peptide, the typical hairpin-like structure comprising two transmembrane helices (Fig. 5B; also [192,193]), and a peripheral domain on the *P*-side of the membrane, which latter can be either CM or ICM. In case of cyanobacterial CcOs, this extrinsic domain has been shown to be uniquely located in two entirely distinct (aqueous) environments, viz. the periplasmic (solvent) space and the thylakoid lumen [45,66] (Fig. 5).

The unusual and unexpected, initially most controversial localization of the cyanobacterial CcO in both of the two osmotically independent membrane systems, viz. CM and ICM (Fig. 3A), was repeatedly corroborated by careful immunological tests [132,194,202,203]. The presence or absence of a binuclear Cu_A centre in this domain is related to the particular nature of haem–copper oxidase in question. Cyanobacterial CcOs use metalloproteins such as Cyt c_6 or PC as (water-soluble) electron donors while QOXs would use lipophilic organic compounds, viz. quinols [177]. In QOXs, the Cu_A centre is missing; yet, the typical central cupredoxin-like fold, formed by a 10-stranded β-barrel, is still conserved. According to the *A*,*B*,*C*-classification [179], which does not account for the specific electron donor, however, Figs. 6 and 7 clearly show that all cyanobacterial haem–copper oxidases are type A_2 oxidases. The latter typically have two helices in the transmembrane part of subunit II [192,193,206]. In *P. denitrificans* CcO the signal sequence of subunit II (residues 1–28) is cleaved off after translocation and the 3D structure of mature *P. denitrificans* CcO subunit II comprises an N-terminal loop (residues 29–53), two transmembrane helices including their connection (residues 54–132 on the *N*-side of the membrane, i.e. "inside"), and a C-terminal globular domain (residues 133–280). Similarly, in cyanobacteria, the signal sequences are most likely cleaved off after translocation leaving the N-terminal loop at the P-side of the membrane, two transmembrane helices including their connection at the N-side of the membrane, and the C-terminal globular domain on the P-side of the membrane (Fig. 5B). Fig. 8 shows amino acid sequences of this structural pattern as found in all cyanobacterial haem–copper oxidases.

Closer inspection of the sequences as well as the models predicting secondary structures reveal significant structural deviations at the N- and C-termini of cyanobacterial haem–copper oxidases as compared to *P. denitrificans* CcO, whereas the core of the soluble domain shows similar structural elements, viz. a β-barrel formed by 10 β-sheets. Subunits II of cyanobacterial haem–copper oxidases display the typical loop located between the second transmembrane helix and the cupredoxin-like domain (ProII-120–GlyII-166, *Synechocystis* numbering), which is not found in *P. denitrificans*, *R. sphaeroides*, *T. thermophilus* [198], or *B. taurus* subunits II nor in *E. coli* QOX subunit II (Fig. 8). This characteristic insertion seems to be another typical feature of haem–copper oxidases of the A_2 subfamily [179]. A peculiarity of the insertion ProII-120–GlyII-166 in subunit II of the *Synechocystis* CcO is its abundance in methionines and histidines (Met131-Ala-His-Asn-His-Met-Gly-His-Met-Gly-Ser-Met-Gly-Asn-Met-Val-Ala-Met146), which does not seem to represent the Cyt *c*-binding site but might be another metal-binding site. Both cyanobacterial CcOs and "QOXs" have this unusual insertion (loop); however, its length and sequence vary. Further specific features of cyanobacterial haem–copper oxidase subunits II are the absence of a region immediately after the potential Cyt *c*-binding site of *P. denitrificans* (AlaII-161–AlaII-185, *Paracoccus* numbering) and the presence of an extended C-terminus (SerII-286–LeuII-332, *Synechocystis* numbering) similar to the QOX from *E. coli* (Fig. 8). At any rate, the functional relevance of these characteristically cyanobacterial features, which were recognized at the time of the first sequencing already [191–194] and were somehow associated with the unusual ionic strength behaviour (Brönsted plots) of PC- and Cyt c_6-oxidizing cyanobacterial CcOs [145–147], is not clear at the moment. Also, if the mere fact that only cyanobacterial but no other known CcOs (*P. denitrificans*, *R. sphaeroides*, and beef heart mitochondrial CcOs were tested so far) could accept PC as electron donor (unpublished observations of the authors) has anything to do with these peculiarities of cyanobacterial CcO remains unknown.

Subunits II of haem–copper oxidases participate in both electron transfer and proton pumping. All cyanobacterial subunits II have in common a glutamate residue (GluII-106 in *P. denitrificans*) located at the beginning of the second transmembrane helix (Fig. 8; Fig. 5B). Since GluII-106 is close to the conserved Lys in the K-channel (see above), it has been suggested to regulate proton entry or transit through this channel [179,180]. In CcOs, electrons donated from either Cyt c_6 or PC enter the oxidase complex via a conserved tryptophan on subunit II (e.g., TrpII-149 in *P. denitrificans*, see Fig. 8), which, when mutated, abolishes electron transfer [204,205]. In the CcO structures published so far [177,187–190,198], this tryptophan is close to the putative Cyt *c*-binding site and molecular modeling has placed the indole ring of this tryptophan within a distance of 4–5 Å from the exposed haem edge of Cyt *c*, which supports its role as the site of electron entry. The corresponding residue of the aligned cyanobacterial CcO subunits II is either a tyrosine, a tryptophan, or–most

interestingly–a phenylalanine. This putative electron entry site on cyanobacterial CcO subunits II is part of a conserved sequence rich in aromatic amino acids (-Tyr-Ala-Trp-Ile-Phe-Thr-Tyr- in *Synechocystis* CcO). Similar to *Paracoccus* CcO, this region could be part of a hydrophobic loop participating in Cyt *c*-binding and facilitating rapid electron transfer.

Finally, electrons are transferred to the Cu_A centre, which is composed of two electronically coupled, mixed-valence copper ions Cu^I/Cu^{II} (oxidized state) [207]. In all known CcOs, this centre seems to be binuclear, which was explained by the unusually low electron transfer reorganization energy at this site, such that electron transfer, proper, is not the rate-limiting step. The ligands for the binuclear Cu_A centre in CcOs are located near the C-terminus. The strictly conserved residues are two His, two Cys, and one Glu and Met, respectively. In *Synechocystis* CcO subunit II, the corresponding ligands are HisII-214, CysII-249, GluII-251, CysII-253, HisII-257, and MetII-260, respectively (Fig. 8; Fig. 5B). By contrast, the Cu_A centre of the QOX is believed to have been lost during evolution from the primeval CcO [181,208]. This follows from inspection of the genes for the "cyanobacterial QOXs": With the exception of CysII-249 and of MetII-260 (*Synechocystis* numbering), these ligands are not found in putative cyanobacterial QOXs. However, the homology between CcOs and (haem–copper) QOXs is underlined by mutational studies on the QOX from *E. coli* where the binuclear binding site in the globular cupredoxin-like domain was functionally restored through genetic manipulation [208].

A structural alignment around the Cu_A centre of *Paracoccus* CcO and the corresponding region of *E. coli* QOX shows that, in true QOXs this site is completely blocked by hydrophobic residues, including TyrII-210 and PheII-215 (*E. coli* numbering), which prevent access from the periplasmic side. Analysis of the hydrophobic region of cyanobacterial subunits II (similarly to that of subunit I; see discussion above; Figs. 7 and 8) does not provide any evidence for a quinol-binding site. Hypothetical amino acid sequences (deduced from genes) of putative cyanobacterial energy-transducing haem–copper quinol oxidases (possibly of bo_3-type as in *E. coli*, see [125,177]) are shown in Figs. 7 and 8. Information about the two better-characterized non-energy-transducing *bd*-type quinol oxidases "I" and "II" (also from *E. coli*), whose (deduced) amino acid sequences of subunits I and II, however, are totally unrelated to haem–copper oxidases (see above), may be found in [182–184].

Regarding a binding site for charged electron donors to cyanobacterial CcOs, the sequence analysis provides some interesting information: The negatively charged patch on subunit II of *Paracoccus* or beef heart mitochondrial CcO seems to be also present on cyanobacterial CcO. In *Paracoccus* CcO AspII-163 and AspII-206 (*Paracoccus* numbering, see Fig. 8) have been shown to be involved in both horse heart Cyt *c*- and bacterial Cyt c_{552}-binding [209]. In cyanobacterial CcO subunits II, only the latter amino acid is conserved (AspII-211 in *Synechocystis*, Fig. 8) because AspII-163 (*Paracoccus* numbering) is part of a region not present in cyanobacteria. Further interactions between the lysines on eukaryotic Cyt *c* and GluII-154 and AspII-187 in *Paracoccus* CcO have been proposed [209] but, interestingly, the corresponding amino acids in cyanobacteria are not acidic (Fig. 8). This might contribute to the unusual ionic strength behaviour (Brönsted plots) of the interaction between *c*-type Cyt's and PCs of various IEPs, and the (membrane-bound) cyanobacterial CcO as was reported previously [140,145–147]. Also, this fits with different physical properties of the endogenous donors in cyanobacteria, viz. the freely interchangeable [137–143] yet abso-

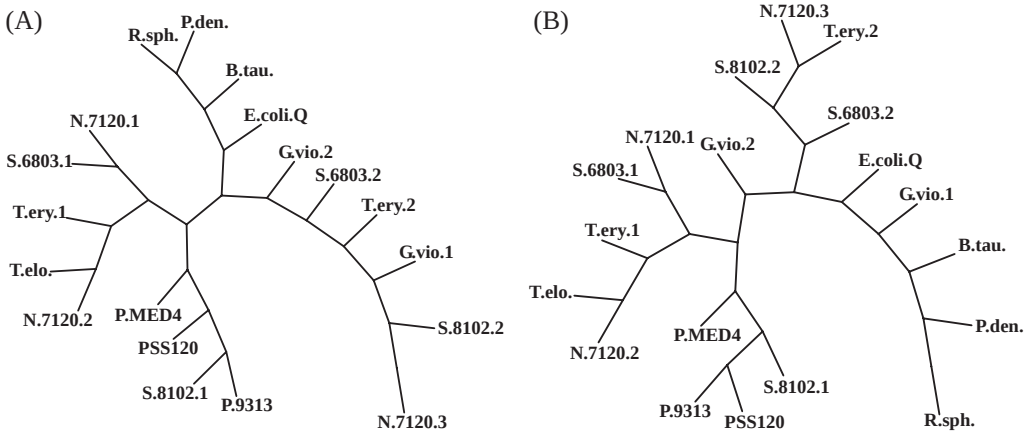


Fig. 9. Unrooted phylogenetic trees of haem–copper oxidase subunits I (A) and subunits II (B). The most likely trees calculated with the programs T-COFFEE, version 1.37 [215], and the PHYLIP package, version 3.57 [214], are shown. After 100 bootstrap cycles with the SEQBOOT subprogram, the data set was subjected to the PROTDIST method of the PHYLIP package where the Dayhoff PAM matrix was formed. This output was subjected to the FITCH phylogenetic method where the search for the best trees was allowed. The produced series of trees were analysed by the CONSENSE method to reveal the most probable tree in each group. Analysed phylogenetic trees were visualized by the program DRAWTREE. Abbreviations of the source organisms are given in Fig. 7 and Table 1.

lutely indispensable [144,145] soluble electron carriers PC and Cyt c_6. The cyanobacterial Cu_A domain is an acidic protein [141] as are, in unicellular species at least, the two in vivo electron donor candidates Cyt c_6 and PC (in marked contrast to the strongly basic mammalian Cyt *c*): IEPs of Cyt c_6 and PC (from *Synechocystis*) are 4.94 and 5.6, respectively. Fig. 9 presents the usual fancy evolutionary "trees" for cyanobacterial CcO subunits I (Fig. 9A) and II (Fig. 9B). Note, however, that the limited value of such "trees" has repeatedly been emphasized particularly when comparing incompatible biochemical sequences during incompatible geological epochs.

Of the non-redox metal ions found in the CcOs so far sequenced (see above; note that *E. coli* QOX [177] did not contain any non-redox metal) the only possible cyanobacterial candidate might be Mg^{2+} since in most, but not all, cyanobacterial sequences (Figs. 7 and 8) HisI-403, AspI-412, and GluII-254 (*Paracoccus* numbering) on the interface between subunits I and II, and on the connecting loop between helices IX and X, respectively, seem to be conserved [154]. However, in a preliminary ICP-AES study on (tiny amounts of) isolated and purified CcOs from *Anacystis* (*Synechococcus*), *Anabaena* (*Nostoc*), and *Synechocystis*, except Fe and Cu (in a molar ratio of 2:3), no other metal could be detected [210].

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References

[1] B.E.J. Pagel, Solar abundances. A new table (October 1976), Phys. Chem. Earth 11 (1979) 79–80.
[2] G.T. Babcock, M. Wikström, Oxygen activation and the conservation of energy in cell respiration, Nature 356 (1992) 301–309.
[3] G. Renger, Apparatus and mechanism of photosynthetic oxygen evolution: a personal perspective, Photosynth. Res. 76 (2003) 269–288.
[4] G. Renger, Coupling of electron and proton transfer in oxidative water cleavage in photosynthesis, Biochim. Biophys. Acta 1655 (2004) 195–204.
[5] J.E. Lennard-Jones, The electronic structure of some diatomic molecules, Trans. Faraday Soc. 25 (1929) 668–674.
[6] W.A. Goddard, B.D. Olafson, Theoretical studies of oxygen binding, Ann. N. Y. Acad. Sci. 367 (1981) 419–433.
[7] N. Lane, Oxygen—The Molecule that Made the World, Oxford University Press, Great Britain, 2003.
[8] V.P. Skulachev, Role of uncoupled and non-coupled oxidations in maintenance of safely low levels of oxygen and its one-electron reductants, Q. Rev. Biophys. 29 (1996) 169–202.
[9] J.G. Morris, The physiology of obligate anaerobiosis, Adv. Microb. Physiol. 12 (1975) 169–246.
[10] J.A. Cole, Microbial gas metabolism, Adv. Microb. Physiol. 14 (1976) 1–84.
[11] E. Cadenas, Biochemistry of oxygen toxicity, Annu. Rev. Biochem. 58 (1989) 79–110.
[12] P. Mitchell, Coupling of phosphorylation to electron and hydrogen transfer by a chemiosmotic type of mechanism, Nature 191 (1961) 144–148.
[13] D.G. Nicholls, S.J. Ferguson, Bioenergetics, vol. 2, Academic Press, London, 1982.
[14] E. Broda, The Evolution of the Bioenergetic Processes, Pergamon Press, Oxford, UK, 1975.
[15] E. Broda, G.A. Peschek, Did respiration or photosynthesis come first? J. Theor. Biol. 81 (1979) 201–212.
[16] J.A. Raven, F.A. Smith, The evolution of chemiosmotic energy coupling, J. Theor. Biol. 57 (1976) 301–312.
[17] G.A. Peschek, E. Broda, Fermentation–prerespiration–photosynthesis–respiration: a bioenergetic succession, in: J.W. Schopf (Ed.), An Interdisciplinary Study of the Origin and Evolution of Earth's Earliest Biosphere, UCLA, Los Angeles, USA, 1980, p. 23.
[18] G.A. Peschek, Phylogeny of photosynthesis and the evolution of electron transport: the bioenergetic backbone, Photosynthetica 15 (1981) 543–554.
[19] C. Obinger, G. Regelsberger, G. Strasser, U. Burner, G.A. Peschek, Purification and characterization of a homodimeric catalase-peroxidase from the cyanobacterium *Anacystis nidulans*, Biochem. Biophys. Res. Commun. 235 (1997) 545–552.
[20] C. Obinger, G. Regelsberger, A. Pircher, A. Sevcik-Klöckler, G. Strasser, G.A. Peschek, Hydrogen peroxide removal in cyanobacteria: characterization of a catalase-peroxidase from *Anacystis nidulans*, in: G.A. Peschek, W. Löffelhardt, G. Schmetterer (Eds.), The Phototrophic Prokaryotes, Kluwer Academic/Plenum Publishers, New York, 1999, pp. 719–731.
[21] G. Regelsberger, C. Obinger, R. Zoder, F. Altmann, G.A. Peschek, Purification and characterization of a hydroperoxidase from the cyanobacterium *Synechocystis* PCC6803: identification of its gene by peptide mass mapping using matrix assisted laser desorption ionization time-of-flight mass spectrometry, FEMS Microbiol. Lett. 170 (1999) 1–12.
[22] G. Regelsberger, C. Jakopitsch, F. Rüker, D. Krois, G.A. Peschek, C. Obinger, Effect of distal cavity mutations on the formation of compound I in catalase-peroxidases, J. Biol. Chem. 275 (2000) 22854–22861.
[23] G. Regelsberger, C. Jakopitsch, L. Plasser, H. Schwaiger, P.G. Furtmüller, G.A. Peschek, M. Zamocky, C. Obinger, Occurrence and biochemistry of hydroperoxidases in oxygenic phototrophic prokaryotes (cyanobacteria), Plant Physiol. Biochem. 40 (2002) 479–490.
[24] C. Jakopitsch, G. Regelsberger, P.G. Furtmüller, G. Rüker, G.A. Peschek, C. Obinger, Engineering the proximal heme cavity of catalase-peroxidase, J. Inorg. Biochem. 91 (2002) 78–86.
[25] W. Atzenhofer, G. Regelsberger, U. Jacob, G.A. Peschek, P.G. Furtmüller, R. Huber, C. Obinger, The 2.0 Å resolution structure of the catalytic portion of a cyanobacterial membrane-bound manganese superoxide dismutase, J. Mol. Biol. 321 (2002) 479–489.
[26] G. Regelsberger, W. Atzenhofer, F. Rüker, G.A. Peschek, C. Jakopitsch, M. Paumann, P.G. Furtmüller, C. Obinger, Biochemical characterization of a membrane-bound manganese-containing superoxide dismutase from the cyanobacterium *Anabaena* 7120, J. Biol. Chem. 277 (2002) 43615–43622.
[27] G. Regelsberger, U. Laaha, D. Ditmann, F. Rüker, A. Canini, M. Grilli-Caiola, P.G. Furtmüller, C. Jakopitsch, G.A. Peschek, C. Obinger, The iron superoxide dismutase from the filamentous

cyanobacterium *Nostoc* PCC 7120: localization, overexpression and biochemical characterization, J. Biol. Chem. 279 (2004) 44384–44393.
[28] J. Castresana, M. Saraste, Evolution of energetic metabolism: the respiration-early hypothesis, Trends Biochem. Sci. 20 (1995) 443–448.
[29] F. Egami, Inorganic types of fermentation and anaerobic respirations in the evolution of energy-yielding metabolism, Orig. Life 5 (1974) 405–409.
[30] D.L. Gilbert (Ed.), Oxygen and Living Processes. An interdisciplinary Approach, Springer Verlag, New York, 1981.
[31] A. Zohner, E. Broda, Urey–Miller experiments on the origin and stability of oxidized nitrogen compounds in a reducing environment, Orig. Life 9 (1979) 291–298.
[32] J. van der Oost, A.P.N. de Boer, J.-W.L. de Gier, W.G. Zumft, A.H. Stouthamer, R.J.M. van Spanning, The heme–copper oxidase family consists of three distinct types of terminal oxidases and is related to nitric oxide reductase, FEMS Microbiol. Lett. 121 (1994) 1–10.
[33] R.A. Scott, W.G. Zumft, C.L. Coyle, D.M. Dooley, *Pseudomonas stutzeri* N_2O reductase contains Cu_A-type sites, Proc. Natl. Acad. Sci. U. S. A. 86 (1989) 4082–4086.
[34] S.L. Miller, H.C. Urey, Organic compound synthesis on the primitive earth, Science 130 (1959) 245–250.
[35] G. Wächtershäuser, Before enzymes and templates: theory of surface metabolism, MicroBiol. Rev. 52 (1988) 452–484.
[36] C. Huber, G. Wächtershäuser, Activated acetic acid by carbon fixation on (Fe,Ni)S under primordial conditions, Science 276 (1997) 245–247.
[37] W. Martin, M.J. Russel, On the origins of cells: a hypothesis for the evolutionary transitions from abiotic geochemistry to chemoautotrophic prokaryotes, and from prokaryotes to nucleated cells, Philos. Trans. R. Soc., B 358 (2003) 59–83.
[38] E.S. Barghoorn, J.W. Schopf, Microorganisms from the late precambrian of Central Australia, Science 150 (1965) 337–339.
[39] E.S. Barghoorn, J.W. Schopf, Microorganisms three billion years old from the precambrian of South Africa, Science 152 (1966) 758–763.
[40] J.W. Schopf, Precambrian microorganisms and evolutionary events prior to the origin of vascular plants, Biol. Rev. 45 (1970) 319–352.
[41] P. Albrecht, G. Ourisson, Biogene Substanzen in Sedimenten und Fossilien, Angew. Chem. 83 (1971) 221–260.
[42] R.H. Crabtree, Where smokers rule, Science 276 (1997) 222.
[43] J.B. Waterbury, S.W. Watson, R.R.L. Guillard, L.E. Brand, Widespread occurrence of a unicellular, marine, planktonic, cyanobacterium, Nature 277 (1979) 293–294.
[44] S.W. Chisholm, R.J. Olson, E.R. Zettler, R. Goericke, J.B. Waterbury, N.A. Welschmeyer, A novel, free living Prochlorophyte abundant in the oceanic euphotic zone, Nature 340 (1988) 340–343.
[45] G.A. Peschek, Photosynthesis and respiration of cyanobacteria: bioenergetic significance and molecular interactions, in: G.A. Peschek, et al., (Eds.), The Phototrophic Prokaryotes, Kluwer Academic/Plenum Publishers, New York, 1999, pp. 201–209.
[46] G.A. Peschek, Cyanobacteria viewed as free living chloromitochondria: the endosymbiont hypothesis revisited, Plant Physiol. Biochem. 38 (2000) 266.
[47] A. Zouni, H.T. Witt, J. Kern, P. Fromme, N. Krauβ, W. Saenger, P. Orth, Crystal structure of photosystem II from *Synechococcus elongatus* at 3.8 Å resolution, Nature 409 (2001) 739–743.
[48] K.N. Ferreira, T.M. Iverson, K. Maghlaoui, J. Barber, S. Iwata, Architecture of the photosynthetic oxygen-evolving center, Science 303 (2004) 1831–1838.
[49] P. Jordan, P. Fromme, H.T. Witt, O. Klukas, W. Saenger, N. Krauß, Three-dimensional structure of cyanobacterial photosystem I at 2.5 Å resolution, Nature 411 (2001) 909–917.
[50] A. Ben-Shem, F. Frolow, N. Nelson, Crystal structure of plant photosystem I, Nature 426 (2003) 630–635.
[51] G. Kurisu, H. Zhang, J.L. Smith, W.A. Cramer, Structure of the cytochrome b_6f complex of oxygenic photosynthesis: tuning the cavity, Science 302 (2003) 1009–1014.
[52] D. Stroebel, Y. Choquet, J.-L. Popot, D. Picot, An atypical haem in the cytochrome b_6f complex, Nature 246 (2003) 413–418.
[53] G.A. Peschek, Structure and function of respiratory membranes in cyanobacteria (blue-green algae), Sub-cell. Biochem. 10 (1984) 85–191.
[54] G.A. Peschek, Respiratory electron transport, in: P. Fay, C. Van Baalen (Eds.), The Cyanobacteria, Elsevier Publishers, Amsterdam, The Netherlands, 1987, pp. 119–171.
[55] G.A. Peschek, C. Obinger, M. Paumann, The respiratory chain of blue-green algae (cyanobacteria), Physiol. Plant. 120 (2004) 358–369.
[56] A. Binder, Respiration and photosynthesis in energy-transducing membranes of cyanobacteria, J. Bioenerg. Biomembranes 14 (1982) 272–286.
[57] H. Koike, K. Satoh, Respiration and photosynthetic electron transport system in cyanobacteria—recent advances, J. Sci. Ind. Res. 55 (1996) 564–582.
[58] O. Holm-Hansen, Ecology, physiology and biochemistry of blue-green algae, Annu. Rev. Microbiol. 21 (1968) 47–70.
[59] T. Bisalputra, D.L. Brown, T.E. Weier, Possible respiratory sites in a blue-green alga *Nostoc sphaericum* as demonstrated by potassium tellurite and tetranitro-blue tetrazolium reduction, J. Ultrastruct. Res. 27 (1969) 182–197.
[60] G.A. Peschek, G. Schmetterer, U.B. Sleytr, Possible respiratory sites in the plasma membrane of *Anacystis nidulans*: ultracytochemical evidence, FEMS Microbiol. Lett. 11 (1981) 121–124.
[61] T. Omata, N. Murata, Cytochromes and prenylquinones in preparations of cytoplasmic and thylakoid membranes from the cyanobacterium (blue-green alga) *Anacystis nidulans*, Biochim. Biophys. Acta 766 (1984) 395–402.
[62] T. Omata, N. Murata, Electron transport reactions in cytoplasmic and thylakoid membranes prepared from the cyanobacteria (blue-green algae) *Anacystis nidulans* and *Synechocystis* PCC6714, Biochim. Biophys. Acta 810 (1985) 354–361.
[63] V. Molitor, G.A. Peschek, Respiratory electron transport in plasma and thylakoid membrane preparations from the cyanobacterium *Anacystis nidulans*, FEBS Lett. 195 (1986) 145–150.
[64] V. Molitor, M. Trnka, G.A. Peschek, Isolated and purified plasma and thylakoid membranes of the cyanobacterium *Anacystis nidulans* contain immunologically cross-reactive aa_3-type cytochrome oxidase, Curr. Microbiol. 14 (1987) 263–268.
[65] G.A. Peschek, Cytochrome oxidase and the *cta* operon of cyanobacteria, Biochim. Biophys. Acta 1275 (1996) 27–32.
[66] G.A. Peschek, Interaction of photosynthesis and respiration, Biochem. Soc. Trans. 24 (1996) 729–733.
[67] G.A. Peschek, Spectral properties of a cyanobacterial cytochrome oxidase: evidence for cytochrome aa_3, Biochem. Biophys. Res. Commun. 98 (1981) 72–79.
[68] G.A. Peschek, Occurrence of cytochrome aa_3 in *Anacystis nidulans*, Biochim. Biophys. Acta 635 (1981) 470–475.
[69] G.A. Peschek, G. Schmetterer, G. Lauritsch, R. Muchl, P.F. Kienzl, W.H. Nitschmann, Do cyanobacteria contain "mammalian-type" cytochrome oxidase? Arch. Microbiol. 131 (1982) 261–265.
[70] M. Wastyn, A. Achatz, M. Trnka, G.A. Peschek, Immunological and spectral characterization of partly purified cytochrome oxidase from the cyanobacterium *Synechocystis* 6714, Biochem. Biophys. Res. Commun. 149 (1987) 102–111.
[71] M. Wastyn, A. Achatz, V. Molitor, G.A. Peschek, Respiratory activities and aa_3-type cytochrome oxidase in plasma and thylakoid membranes from vegetative cells and heterocysts of the cyanobacterium *Anabaena* ATCC 29413, Biochim. Biophys. Acta 935 (1988) 224–317.
[72] M. Trnka, G.A. Peschek, Immunological identification of aa_3-type cytochrome oxidase in membrane preparations of the cyanobacte-

rium *Anacystis nidulans*, Biochem. Biophys. Res. Commun. 136 (1986) 235–241.
[73] G.A. Peschek, V. Molitor, M. Trnka, M. Wastyn, W. Erber, Characterization of cytochrome-*c* oxidase in isolated and purified plasma and thylakoid membranes from cyanobacteria, Methods Enzymol. 167 (1988) 437–449.
[74] G.A. Peschek, M. Wastyn, M. Trnka, V. Molitor, I.V. Fry, L. Packer, Characterization of the cytochrome-*c* oxidase in isolated and purified plasma membranes from the cyanobacterium *Anacystis nidulans*, Biochemistry 28 (1989) 3057–3063.
[75] G.A. Peschek, B. Hinterstoisser, M. Wastyn, O. Kuntner, B. Pineau, A. Missbichler, J. Lang, Chlorophyll precursors in the plasma membrane of a cyanobacterium, *Anacystis nidulans*, J. Biol. Chem. 264 (1989) 11827–11832.
[76] G.A. Peschek, M. Wastyn, V. Molitor, H. Kraushaar, C. Obinger, H.C.P. Matthijs, Self-contained or accessory respiration in the phototrophic cyanobacteria (blue-green algae)? in: A. Kotyk, J. Skoda, V. Paces, V. Kosta (Eds.), Highlights of Modern Biochemistry, VSP Intern. Sci. Publishers, Zeist, The Netherlands, 1989, pp. 893–902.
[77] G.A. Peschek, The physiological relevance of cyanobacterial respiration, Plant Cell Physiol. 41 (2000) 14.
[78] J. Oelze, G. Drews, Membranes of photosynthetic bacteria, Biochim. Biophys. Acta 265 (1972) 209–239.
[79] R. Douce, M.A. Block, A.-J. Dorne, J. Joyard, The plastid envelope membranes: their structure, composition and role in chloroplast biogenesis, Sub-cell. Biochem. 10 (1984) 1–84.
[80] J. Oelze, Composition and development of the bacterial photosynthetic apparatus, Sub-cell. Biochem. 8 (1981) 1–73.
[81] G. Schäfer, M. Engelhard, V. Müller, Bioenergetics of the Archaea, Microbiol. Mol. Biol. Rev. 63 (1999) 570–620.
[82] G.A. Peschek, Reduced sulfur and nitrogen compounds and molecular hydrogen as electron donors for anaerobic CO_2 photoreduction in *Anacystis nidulans*, Arch. Microbiol. 119 (1978) 313–322.
[83] G.A. Peschek, Anaerobic hydrogenase activity in *Anacystis nidulans*. H_2-dependent photoreduction and related reactions, Biochim. Biophys. Acta 548 (1979) 187–202.
[84] G.A. Peschek, Aerobic hydrogenase activity in *Anacystis nidulans.* The oxyhydrogen reaction, Biochim. Biophys. Acta 548 (1979) 203–215.
[85] G.A. Peschek, Evidence for two functionally distinct hydrogenases in *Anacystis nidulans*, Arch. Microbiol. 123 (1979) 81–92.
[86] G.A. Peschek, The role of the Calvin cycle for anoxygenic CO_2 photoassimilation in *Anacystis nidulans*, FEBS Lett. 106 (1979) 34–38.
[87] O. Schmitz, G. Boison, R. Hilscher, B. Hundeshagen, W. Zimmer, F. Lottspeich, H. Bothe, Molecular biological analysis of a bidirectional hydrogenase from cyanobacteria, Eur. J. Biochem. 233 (1995) 266–276.
[88] P. Houchins, The physiology and biochemistry of hydrogen metabolism in cyanobacteria, Biochim. Biophys. Acta 768 (1984) 227–255.
[89] H. Bothe, G. Boison, O. Schmitz, Hydrogenases in cyanobacteria, in: G.A. Peschek, et al., (Eds.), The Phototrophic Prokaryotes, Kluwer Academic/Plenum Publishers, New York, 1999, pp. 589–601.
[90] P. Tamagnini, R. Axelssson, P. Lindberg, F. Oxelfelt, R. Wünschiers, P. Lindblad, Hydrogenases and hydrogen metabolism of cyanobacteria, Microbiol. Mol. Biol. Rev. 66 (2002) 1–20.
[91] B.J. Jensen, Energy requirement for diazotrophic growth of the cyanobacterium *Anabaena variabilis* determined from growth yields in the dark, J. Gen. Microbiol. 129 (1983) 2633–2640.
[92] H. Gest, Bioenergetic and metabolic process patterns in anoxyphototrophs, in: G.A. Peschek, et al., (Eds.), The Phototrophic Prokaryotes, Kluwer Academic/Plenum Publishers, 1999, pp. 11–19.
[93] E. Broda, G.A. Peschek, Evolutionary considerations on the thermodynamics of nitrogen fixation, BioSystems 13 (1980) 47–56.
[94] E. Broda, G.A. Peschek, Nitrogen fixation as evidence for the reducing nature of the early biosphere, BioSystems 16 (1983) 1–8.
[95] E. Broda, G.A. Peschek, The evolution of dinitrogen fixation, Orig. Life 14 (1984) 653–656.
[96] S. Berger, U. Ellersiek, K. Steinmüller, Cyanobacteria contain a mitochondrial complex I-homologous NADH-dehydrogenase, FEBS Lett. 286 (1991) 129–132.
[97] T. Friedrich, K. Steinmüller, H. Weiss, The proton-pumping respiratory complex I of bacteria and mitochondria and its homologue in chloroplasts, FEBS Lett. 367 (1995) 107–111.
[98] H. Weiss, T. Friedrich, G. Hofhaus, D. Preis, The respiratory-chain NADH dehydrogenase (complex I) of mitochondria, Eur. J. Biochem. 197 (1991) 563–576.
[99] T. Yagi, The bacterial energy-transducing NADH-quinone oxidoreductases, Biochim. Biophys. Acta 1141 (1993) 1–17.
[100] T. Friedrich, H. Weiss, Modular evolution of the respiratory NADH: ubiquinone oxidoreductase and the origin of its modules, J. Theor. Biol. 187 (1997) 529–540.
[101] T. Friedrich, B. Böttcher, The gross structure of the respiratory complex I: a Lego System, Biochim. Biophys. Acta 1608 (2004) 1–9.
[102] H. Leif, V.D. Sled, T. Ohnishi, H. Weiss, T. Friedrich, Isolation and characterization of the proton-translocating NADH: ubiquinone oxidoreductase from *Escherichia coli*, Eur. J. Biochem. 230 (1995) 538–548.
[103] G. Boison, H. Bothe, A. Hansel, P. Lindblad, Evidence against a common use of the diaphorase subunits by the bidirectional hydrogenase and by the respiratory complex I in cyanobacteria, FEMS Microbiol. Lett. 174 (1999) 159–165.
[104] I. Alpes, S. Scherer, P. Böger, The respiratory NADH dehydrogenase of the cyanobacterium *Anabaena variabilis*: purification and characterization, Biochim. Biophys. Acta 973 (1989) 41–46.
[105] W. Cooley, C.A. Howitt, W.F.J. Vermaas, Succinate:quinol oxidoreductases in the cyanobacterium *Synechocystis* sp. strain 6803: presence and function in metabolism and electron transport, J. Bacteriol. 182 (2000) 714–722.
[106] W. Cooley, W.F.J. Vermaas, Succinate dehydrogenase and other respiratory pathways in thylakoid membranes of *Synechocystis* sp. strain PCC 6803: capacity comparisons and physiological function, J. Bacteriol. 183 (2001) 4251–4258.
[107] H. Ohkawa, H.B. Pakrasi, T. Ogawa, Two types of functionally distinct NAD(P)H dehydrogenases in *Synechocystis* sp. strain PCC 6803, J. Biol. Chem. 275 (2000) 31630–31634.
[108] V.A. Dzelzkalns, C. Obinger, G. Regelsberger, H. Niederhauser, M. Kamensek, G.A. Peschek, L. Bogorad, Deletion of the structural gene for the NADH-dehydrogenase subunit 4 of *Synechocystis* 6803 alters respiratory properties, Plant Physiol. 105 (1994) 1435–1442.
[109] H. Ohkawa, G.D. Price, M.R. Badger, T. Ogawa, Mutation of ndh genes leads to inhibition of CO_2 uptake rather than HCO_3 uptake in *Synechocystis* sp. strain PCC 6803, J. Bacteriol. 182 (2000) 2591–2596.
[110] C. Hägerhäll, Succinate:quinone oxidoreductases—variations on a conserved theme, Biochim. Biophys. Acta 1320 (1997) 107–141.
[111] C.R.D. Lancaster, *Wolinella succinogenes* quinol: fumarate reductase and its comparison to *E. coli* succinate:quinone reductase, FEBS Lett. 555 (2003) 21–28.
[112] C.R.D. Lancaster, *Wolinella succinogenes* quinol:fumarate reductase-2.2-Å-resolution crystal structure and the E-pathway hypothesis of coupled transmembrane proton and electron transfer, Biochim. Biophys. Acta 1665 (2002) 215–231.
[113] C.R.D. Lancaster, A. Kröger, M. Auer, H. Michel, Structure of fumarate reductase from *Wolinella succinogenes* at 2.2-Å-resolution, Nature 402 (1999) 377–385.
[114] V. Yankovskaya, R. Horsefield, S. Törnroth, C. Lunba-Chavez, H. Miyoshi, C. Leger, B. Byrne, G. Cecchini, S. Iwata, Architecture of

succinaste dehydrogenase and reactive oxygen species generation, Science 299 (2003), 700–704.
[115] H. Flasch, Spectrophotometric investigations on complexes I, II and III of the respiratory electron transport system in isolated and purified plasma and thylakoid membranes of cyanobacteria, Diploma thesis, University of Vienna, Austria, 1997.
[116] S. Scherer, I. Alpes, H. Sadowski, P. Böger, Ferredoxin-$NADP^+$ oxidoreductase is the respiratory NADPH dehydrogenase of the cyanobacterium *Anabaena variabilis*, Arch. Biochem. Biophys. 267 (1988) 228–235.
[117] T. Festetics, Kinetic investigations on the oxidation of succinate and NAD(P)H by plasma and thylakoid membranes isolated and purified from cyanobacteria, Diploma thesis, University of Vienna, Austria, 2004.
[118] W. Gray, W.F. Doolittle, Has the endosymbiont hypothesis been proven? MicroBiol. Rev. 46 (1982) 1–42.
[119] G.A. Peschek, Restoration of respiratory electron transport reactions in quinone-depleted particle preparations from *Anacystis nidulans*, Biochem. J. 186 (1980) 515–523.
[120] G.A. Peschek, O. Kuntner, Differential effects of plastoquinone and phylloquinone in photosynthetic and respiratory electron transfer reactions of *n*-pentane-extracted membrane preparations from cyanobacteria, Photobiochem. Photobiophys. Suppl. (1987) 157–166.
[121] G.A. Peschek, M. Wastyn, S. Fromwald, B. Mayer, Occurrence of heme O in photoheterotrophically growing, semi-anaerobic cyanobacterium *Synechocystis* sp. PCC6803, FEBS Lett. 371 (1995) 89–93.
[122] G.A. Peschek, D. Alge, S. Fromwald, B. Mayer, Transient accumulation of heme *O* (cytochrome *o*) in the cytoplasmic membrane of semi-anaerobic *Anacystis nidulans*, J. Biol. Chem. 270 (1995) 27937–27941.
[123] G. Auer, B. Mayer, M. Wastyn, S. Fromwald, K. Eghbalzad, D. Alge, G.A. Peschek, Promiscuity of heme groups in the cyanobacterial cytochrome-*c* oxidase, Biochem. Mol. Biol. Int. 37 (1995) 1173–1185.
[124] S. Fromwald, R. Zoder, M. Wastyn, M. Lübben, G.A. Peschek, Extended heme promiscuity in the cyanobacterial cytochrome *c* oxidase: characterization of native complexes containing hemes *A*, *O*, and *D*, respectively, Arch. Biochem. Biophys. 367 (1999) 122–128.
[125] C.A. Howitt, W.F.J. Vermaas, Quinol and cytochrome oxidases in the cyanobacterium *Synechocystis* sp. PCC 6803, Biochemistry 37 (1998) 17944–17951.
[126] N. Sone, G. Sawa, T. Sone, S. Noguchi, Thermophilic bacilli have split cytochrome *b* genes for cytochrome b_6 and subunit IV, J. Biol. Chem. 270 (1995) 10612–10617.
[127] E.A. Berry, M. Guergova-Kuras, L. Huang, A.R. Crofts, Structure and function of cytochrome *bc* complexes, Annu. Rev. Biochem. 69 (2000) 1005–1075.
[128] R. Malkin, Cytochrome bc_1 and b_6f-complexes of photosynthetic membranes, Photosynth. Res. 33 (1992) 121–136.
[129] B.L. Trumpower, Cytochrome bc_1 complexes of microorganisms, MicroBiol. Rev. 54 (1990) 101–129.
[130] B.L. Trumpower, The protonmotive Q cycle, J. Biol. Chem. 265 (1990) 11409–11412.
[131] H. Kraushaar, S. Hager, M. Wastyn, G.A. Peschek, Immunologically cross-reactive and redox-competent cytochrome b_6f-complexes in the chlorophyll-free plasma membrane of cyanobacteria, FEBS Lett. 273 (1990) 227–231.
[132] D.M. Sherman, T.A. Troyan, L.A. Sherman, Localization of membrane proteins in the cyanobacterium *Synechococcus* sp. PCC7942, Plant Physiol. 106 (1994) 251–262.
[133] G. Hauska, W. Nitschke, R.G. Herrmann, Amino acid identities in the three redox center-carrying polypeptides of cytochrome bc_1/b_6f complexes, J. Bioenerg. Biomembranes 20 (1988) 211–228.
[134] A. Dworsky, B. Mayer, G. Regelsberger, S. Fromwald, G.A. Peschek, Functional and immunological characterization of both 'mitochondria-like' and 'chloroplast-like' electron/proton transport proteins in isolated and purified cyanobacterial membranes, Bioelectrochem. Bioenerg. 38 (1995) 35–43.
[135] C. Klughammer, V. Hager, E. Padan, W. Schütz, U. Schreiber, Y. Shahak, G. Hauska, Reduction of cytochromes with menaquinol and sulfide in membranes from green sulfur bacteria, Photosynth. Res. 43 (1995) 27–34.
[136] W. Nitschke, B. Schoepp, B. Floss, A. Schricker, A.W. Rutherford, U. Liebl, Membrane-bound *c*-type cytochromes in *Heliobacillus mobilis*. Characterization by EPR and optical spectroscopy in membranes and detergent-solubilised material, Eur. J. Biochem. 242 (1996) 695–702.
[137] G. Sandmann, P. Böger, Copper-induced exchange of plastocyanin and cytochrome *c*-553 in cultures of *Anabaena variabilis*, Plant Sci. Lett. 17 (1980) 417–424.
[138] H. Siegelman, I.R. Rasched, K.-J. Kunert, P. Kroneck, P. Böger, Plastocyanin: possible significance of quaternary structure, Eur. J. Biochem. 64 (1976) 131–140.
[139] W. Lockau, Evidence for a dual role of cytochrome *c*-553 and plastocyanin in photosynthesis and respiration of the cyanobacterium, *Anabaena variabilis*, Arch. Microbiol. 128 (1981) 336–340.
[140] P.R. Peikert, Plastocyanin from *Synechocystis* 6803-isolation, purification, properties and the role of ionic strength in electron transfer to cyanobacterial membranes, Diploma thesis, University of Vienna, Austria, 1995.
[141] M. Paumann, B. Lubura, G. Regelsberger, M. Feichtinger, G. Köllensberger, C. Jakopitsch, P.G. Furtmüller, C. Obinger, G.A. Peschek, Soluble Cu_A domain of cyanobacterial cytochrome *c* oxidase, J. Biol. Chem. 27 (2004) 10293–10303.
[142] M. Paumann, M. Feichtinger, M. Bernroitner, J. Goldfuhs, C. Jakopitsch, P.G. Furtmüller, G. Regelsberger, G.A. Peschek, C. Obinger, Kinetics of interprotein electron transfer between cytochrome c_6 and the soluble Cu_A domain of cyanobacterial cytochrome *c* oxidase, FEBS Lett. 576 (2004) 101–106.
[143] M. Paumann, M. Bernroitner, B. Lubura, M. Peer, C. Jakopitsch, P.G. Furtmüller, G.A. Peschek, C. Obinger, Kinetics of electron transfer between plastocyanin and the soluble Cu_A domain of cyanobacterial cytochrome *c* oxidase, FEMS Microbiol. Lett. 239 (2004) 301–307.
[144] V. Duran, M. Hervas, M.A. De la Rosa, J.A. Navarro, The efficient functioning of photosynthesis and respiration in *Synechocystis* sp. 6803 strictly requires the presence of either cytochrome c_6 or plastocyanin, J. Biol. Chem. 279 (2004) 7229–7233.
[145] D. Moser, P. Nicholls, M. Wastyn, G.A. Peschek, Acidic cytochrome c_6 of unicellular cyanobacteria is an indispensable and kinetically competent electron donor to cytochrome oxidase in plasma and thylakoid membranes, Biochem. Int. 24 (1991) 757–768.
[146] P. Nicholls, C. Obinger, H. Niederhauser, G.A. Peschek, Cytochrome *c* and *c*-554 oxidation by membranous *Anacystis nidulans* cytochrome oxidase, Biochem. Soc. Trans. 19 (1991) 252S.
[147] P. Nicholls, C. Obinger, H. Niederhauser, G.A. Peschek, Cytochrome oxidase in *Anacystis nidulans*: stoichiometries and possible functions in cytoplasmic and thylakoid membranes, Biochim. Biophys. Acta 1098 (1992) 184–190.
[148] B.G. Schlarb-Ridley, D.S. Bendall, C.J. Howe, Role of electrostatics in the interaction between cytochrome *f* and plastocyanin of the cyanobacterium *Phormidium laminosum*, Biochemistry 41 (2002) 3279–3285.
[149] B.G. Schlarb-Ridley, D.S. Bendall, C.J. Howe, Relation between interface properties and kinetics of electron transfer in the interaction of cytochrome *f* and plastocyanin from plants and the cyanobacterium *Phormidium laminosum*, Biochemistry 42 (2003) 4057–4063.
[150] B.G. Schlarb-Ridley, J.A. Navarro, M. Spencer, D.S. Bendall, M. Hervas, C.J. Howe, M.A. De la Rosa, Role of electrostatics in the interaction between plastocyanin and photosystem I of the cyano-

bacterium *Phormidium laminosum*, Eur. J. Biochem. 269 (2002) 5893–5902.
[151] A. Marcus, On the theory of oxidation–reduction reactions involving electron transfer, J. Chem. Phys. 24 (1956) 966–979.
[152] L. Zhang, H.B. Pakrasi, J. Whitmarsh, Photoautotrophic growth of the cyanobacterium *Synechocystis* sp. PCC6803 in the absence of cytochrome *c*-553 and plastocyanin, J. Biol. Chem. 269 (1994) 5036–5042.
[153] J.A. Garcia-Horsman, B. Barquera, J. Rumbley, J. Ma, R.B. Gennis, The superfamily of heme–copper respiratory oxidases, J. Bacteriol. 176 (1994) 5587–5600.
[154] S. Ferguson-Miller, G.T. Babcock, Heme/copper terminal oxidases, Chem. Rev. 96 (1996) 2889–2907.
[155] M. Wikström, Proton pump coupled to cytochrome *c* oxidase in mitochondria, Nature 266 (1977) 271–273.
[156] J.P. Abrahams, A.G.W. Leslie, R. Lutter, J.E. Walker, Structure at 2.8 Å resolution of F1-ATPase from bovine heart mitochondria, Nature 370 (1994) 621–628.
[157] G.A. Peschek, T. Czerny, W.H. Nitschmann, Respiratory proton extrusion and plasma membrane energization, Methods Enzymol. 167 (1988) 361–379.
[158] B.A. Haddock, C.W. Jones, Bacterial respiration, Bacteriol. Rev. 41 (1977) 47–99.
[159] M.J.S. Kelly, R.K. Poole, M.G. Yates, C. Kennedy, Cloning and mutagenesis of genes encoding the cytochrome *bd* terminal oxidase complex in *Azotobacter vinelandii*: mutants deficient in the cytochrome *d* complex are unable to fix nitrogen in air, J. Bacteriol. 172 (1990) 6010–6019.
[160] R. D'mello, S. Palmer, S. Hill, R.K. Poole, The cytochrome *bd* terminal oxidase of *Azotobacter vinelandii*: low temperature photodissociation spectrophotometry reveals reactivity of cytochrome *b*595 and *d* with carbon monoxide and oxygen, FEMS Microbiol. Lett. 121 (1994) 115–120.
[161] M.G. Yates, C.W. Jones, Respiration and nitrogen fixation in *Azotobacter*, Adv. Microb. Physiol. 11 (1974) 97–135.
[162] G.A. Peschek, K. Villgratter, M. Wastyn, 'Respiratory protection' of the nitrogenase in dinitrogen-fixing cyanobacteria, Plant Soil 137 (1991) 17–24.
[163] G.A. Peschek, E. Broda, Utilization of fructose by a unicellular blue-green alga, *Anacystis nidulans*, Naturwissenschaften 60 (1973) 479–480.
[164] L.J. Stal, R. Moezelaar, Fermentation in cyanobacteria, FEMS MicroBiol. Rev. 21 (1997) 179–211.
[165] G.A. Peschek, R. Zoder, Temperature stress and basic bioenergetic strategies for stress defence, in: L.C. Rai, J.P. Gaur (Eds.), Algal Adaptation to Environmental Stresses: Physiological, Biochemical and Molecular Mechanisms, Springer Verlag, Berlin-Heidelberg, 2001, pp. 203–258.
[166] N. Sone, Y. Fujiwara, Haem O_2 can replace haem A in the active site of cytochrome *c* oxidase from thermophilic bacterium PS3, FEBS Lett. 288 (1991) 154–158.
[167] A. Puustinen, J.E. Morgan, M. Verkhovsky, J.W. Thomas, R.B. Gennis, M. Wikström, The low-spin heme site of cytochrome *o* of *Escherichia coli* is promiscuous with respect to heme type, Biochemistry 31 (1992) 10363–10369.
[168] K. Matsushita, H. Ebisuya, O. Adachi, Homology in the structure and the prosthetic groups between two different terminal ubiquinol oxidases, cytochrome a_1 and cytochrome *o*, of *Acetobacter aceti*, J. Biol. Chem. 267 (1992) 24748–24753.
[169] M. Lübben, J. Morand, Novel prenylated hemes as cofactors of cytochrome oxidases, J. Biol. Chem. 269 (1994) 21473–21479.
[170] M. Lübben, Cytochromes of archaeal electron transfer chains, Biochim. Biophys. Acta 1229 (1994) 1–22.
[171] R.J. Porra, W. Schäfer, E. Cmiel, I. Katheder, H. Scheer, Derivation of the formyl-group oxygen of chlorophyll *b* from molecular oxygen in greening leaves of a higher plant (*Zea mays*), FEBS Lett. 323 (1993) 31–34.
[172] D.P. Jones, Hypoxia and drug metabolism, Biochem. Pharmacol. 30 (1981) 1019–1023.
[173] P. Nicholls, J.M. Wrigglesworth, Routes of cytochrome a_3 reduction. The neoclassical model revisited, Ann. N.Y. Acad. Sci. 550 (1988) 59–67.
[174] C.E. Cooper, P. Nicholls, J.A. Freedman, Cytochrome *c* oxidase: structure, function, and membrane topology of the polypeptide subunits, Biochem. Cell. Biol. 69 (1991) 586–607.
[175] P. Nicholls, A Darwinian View of Cytochromes and Channels, Evolutionary Biology, Principles of Medical Biology, vol. 1B, JAI Press, 1994, pp. 235–251.
[176] P. Nicholls, The mitochondrial and bacterial respiratory chains: from MacMunn and Keilin to current concepts, in: S. Papa (Ed.), Frontiers of Cellular Bioenergetics, Kluwer Academic/Plenum Publishers, New York, 1999, pp. 1–22.
[177] J. Abramson, S. Riistama, G. Larsson, A. Jasaitis, M. Svensson-Ek, L. Laakkonen, A. Puustinen, M. Wikström, The structure of the ubiquinol oxidase from *E. coli* and its ubiquinone binding site, Nat. Struct. Biol. (2000) 910–917.
[178] J. Abramson, M. Svensson-Ek, B. Byrne, S. Iwata, Structure of cytochrome *c* oxidase: a comparison of the bacterial and mitochondrial enzymes, Biochim. Biophys. Acta 1544 (2001) 1–9.
[179] M.M. Pereira, M. Santana, M. Teixeira, A novel scenario for the evolution of haem–copper oxygen reductases, Biochim. Biophys. Acta 1505 (2001) 185–208.
[180] M.M. Pereira, C.M. Gomes, M. Teixeira, Plasticity of proton pathways in haem–copper oxygen reductases, FEBS Lett. 522 (2002) 14–18.
[181] G.A. Peschek, H. Niederhauser, C. Obinger, Cyanobacterial cytochrome oxidase: a candidate for the primordial enzyme? EBEC Short Rep. 7 (1992) 48.
[182] A. Hata-Tanaka, K. Matsuura, S. Itoh, Y. Anraku, Electron flow and heme–heme interaction between cytochromes *b*-558, *b*-595 and d in a terminal oxidase of *Escherichia coli*, Biochim. Biophys. Acta 893 (1987) 289–295.
[183] G.N. Green, H. Fang, R.J. Lin, G. Newton, M. Mather, C.D. Georgiou, R.B. Gennis, The nucleotide sequence of the cyd locus encoding the two subunits of the cytochrome *d* terminal oxidase complex of *Escherichia coli*, J. Biol. Chem. 263 (1988) 13138–13143.
[184] J. Dassa, H. Fsihi, C. Marck, M. Dion, M. Kieffer-Bontemps, P.L. Boquet, A new oxygen-regulated operon in *Escherichia coli* comprises the gene for a putative third cytochrome oxidase and for pH 2.5 acid phosphatase (appA), Mol. Gen. Genet. 229 (1991) 341–352.
[185] P. Nicholls, The biology of the water molecule, Cell. Mol. Life Sci. 57 (2000) 987–992.
[186] J. Barber, Water, water everywhere, and its remarkable chemistry, Biochim. Biophys. Acta 1655 (2004) 123–132.
[187] S. Iwata, C. Ostermeier, B. Ludwig, H. Michel, Structure at 2.8 Å resolution of cytochrome *c* oxidase from *Paracoccus denitrificans*, Nature 376 (1995) 660–669.
[188] M. Svensson-Ek, J. Abramson, G. Larsson, S. Törnroth, P. Brzesinski, S. Iwata, The X-ray crystal structures of wild-type and EQ(I-286) mutant cytochrome *c* oxidases from *Rhodobacter sphaeroides*, J. Mol. Biol. 321 (2002) 329–339.
[189] A. Tsukihara, H. Aoyama, E. Yamashita, T. Tomizaki, H. Yamaguchi, K. Shinzawa-Itoh, R. Nakashima, R. Yaono, S. Yoshikawa, Structures of metal sites of oxidized bovine heart cytochrome *c* oxidase at 2.8 Å, Science 269 (1995) 1069–1074.
[190] T. Tsukihara, H. Aoyama, E. Yamashita, T. Tomizaki, H. Yamaguchi, K. Shinzawa-Itoh, R. Nakashima, R. Yaono, S. Yoshikawa, The whole structure of the 13-subunit oxidized cytochrome *c* oxidase at 2.8 Å, Science 272 (1996) 1136–1144.
[191] D. Alge, Cytochrome oxidase genes of the cyanobacterium *Synechocystis* PCC 6803, Doctoral thesis, University of Vienna, Austria, 1990.
[192] D. Alge, G.A. Peschek, Characterization of a *cta*/*CDE* operon-like genomic region encoding subunits I–III of the cytochrome *c* oxidase

of the cyanobacterium *Synechocystis* PCC 6803, Biochem. Mol. Biol. Int. 29 (1993) 511–525.
[193] D. Alge, G.A. Peschek, Identification and characterization of the *ctaC* (*coxB*) gene as part of an operon encoding subunits I, II, and III of the cytochrome *c* oxidase (cytochrome aa_3) in the cyanobacterium *Synechocystis* PCC 6803, Biochem. Biophys. Res. Commun. 191 (1993) 9–17.
[194] D. Alge, G. Schmetterer, G.A. Peschek, The gene encoding cytochrome-*c* oxidase subunit I from *Synechocystis* PCC 6803, Gene 138 (1994) 127–132.
[195] N. Sone, H. Tano, M. Ishizuka, The genes in the thermophilic cyanobacterium *Synechococcus vulcanus* encoding cytochrome-*c* oxidase, Biochim. Biophys. Acta 1183 (1993) 130–138.
[196] S. Riistama, A. Puustinen, A. Garcia-Horsman, S. Iwata, H. Michel, M. Wikström, Channeling dioxygen into the respiratory enzyme, Biochim. Biophys. Acta 1275 (1996) 1–4.
[197] G. Buse, T. Soulimane, M. Dewor, H.E. Meyer, M. Blüggel, Evidence for a copper-coordinated histidine-tyrosine cross-link in the active site of cytochrome oxidase, Protein Sci. 8 (1999) 985–990.
[198] T. Soulimane, G. Buse, G.P. Burenkov, H.T. Bartunik, R. Huber, M.E. Than, Structure and mechanism of the aberrant ba_3 cytochrome *c* oxidase from *Thermus thermophilus*, EMBO J. 19 (2000) 1766–1776.
[199] S. Siletskiy, T. Soulimane, N. Azarkina, T.V. Wygodina, G. Buse, A. Kaulen, A. Konstantinov, Time resolved generation of a membrane potential by ba_3 cytochrome *c* oxidase from *Thermus thermophilus*. Evidence for reduction induced opening of the binuclear center, FEBS Lett. 457 (1999) 98–102.
[200] C. Backgren, G. Hummer, M. Wikström, A. Puustinen, Proton translocation by cytochrome *c* oxidase can take place without the conserved glutamic acid in subunit I, Biochemistry 39 (2000) 7863–7867.
[201] K.A. Gray, M. Grooms, H. Mylikallio, C. Moomaw, C. Slaughter, F. Daldal, *Rhodobacter capsulatus* contains a novel *cb* type cytochrome *c* oxidase without a Cu_A center, Biochemistry 33 (1994) 3120–3127.
[202] G.A. Peschek, C. Obinger, D.M. Sherman, L.A. Sherman, Immunocytochemical localization of the cytochrome *c* oxidase in a cyanobacterium, *Synechococcus* PCC7942 (*Anacystis nidulans*), Biochim. Biophys. Acta 1187 (1994) 369–372.
[203] G.A. Peschek, C. Obinger, S. Fromwald, B. Bergman, Correlation between immuno-gold labels and activities of the cytochrome-*c* oxidase (aa_3-type) in membranes of salt stressed cyanobacteria, FEMS Microbiol. Lett. 124 (1994) 431–438.
[204] H. Witt, F. Malatesta, F. Nicoletti, M. Brunori, B. Ludwig, Tryptophan 121 of subunit II is the electron entry site to cytochrome *c* oxidase in *Paracoccus denitrificans*. Involvement of a hydrophobic patch in the docking reaction, J. Biol. Chem. 273 (1998) 5132–5136.
[205] Y. Zhen, C.W. Hoganson, G.T. Babcock, S. Ferguson-Miller, Definition of the interaction domain for cytochrome *c* on cytochrome *c* oxidase. I. Biochemical, spectral, and kinetic characterization of surface mutants in subunit II of *Rhodobacter sphaeroides* cytochrome aa_3, J. Biol. Chem. 274 (1999) 38032–38041.
[206] D. Alge, M. Wastyn, C. Mayer, C. Jungwirth, U. Zimmermann, R. Zoder, S. Fromwald, G.A. Peschek, Allosteric properties of cyanobacterial cytochrome *c* oxidase: inhibition of the coupled enzyme by ATP and stimulation by ADP, IUBMB Life 48 (1999) 187–197.
[207] F. Malatesta, F. Nicoletti, V. Zickermann, B. Ludwig, M. Brunori, Electron entry in a Cu_A mutant of cytochrome *c* oxidase from *Paracoccus denitrificans*. Conclusive evidence on the initial electron entry metal center, FEBS Lett. 434 (1998) 322–324.
[208] M. Wilmanns, P. Lappalainen, M. Kelly, E. Sauer-Eriksson, M. Saraste, Crystal structure of the membrane-exposed domain from a respiratory quinol oxidase complex with an engineered dinuclear copper center, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 11949–11951.
[209] V. Drosou, B. Reincke, M. Schneider, B. Ludwig, Specificity of the interaction between the *Paracoccus denitrificans* oxidase and its substrate cytochrome *c*: comparing the mitochondrial to the homologous bacterial cytochrome *c*(552), and its truncated and site-directed mutants, Biochemistry 41 (2002) 10629–10634.
[210] G.A. Peschek, H. Niederhauser, C. Obinger, Metal content of the cytochrome *c* oxidase (aa_3-type) from cyanobacteria, Abstracts 11th Intern. Biophysics Congress, Budapest, Hungary 1993, p. 201.
[211] G.M. Rutten, Geologic data on atmospheric history, Palaeogeogr. Palaeoclimatol. Palaeoecol. 2 (1966) 47–57.
[212] M. Schidlowski, Probleme der atmosphärischen Evolution im Präkambrium, Geol. Rundsch. 60 (1971) 1351–1384.
[213] D.C. Rhoads, J.W. Morse, Evolutionary and geological significance of oxygen-deficient marine basins, Lethaia 4 (1970) 413–428.
[214] J. Felsenstein, Inferring phylogenesis from protein sequences by parsimony, distance, and likelihood methods, Methods Enzymol. 266 (1996) 418–427.
[215] C. Notredame, J. Higgins, J. Heringa, T-Coffee: a novel method for fast and accurate multiple sequence alignment, J. Mol. Biol. 302 (2000) 205–217.
[216] G.A. Peschek, G. Schmetterer, H. Wagesreiter, Oxidation of exogenous *c*-type cytochromes by intact spheroplasts of *Anacystis nidulans*, Arch. Microbiol. 133 (1982) 222–224.
[217] G.A. Peschek, G. Schmetterer, G. Lauritsch, R. Muchl, P.F. Kienzl, W.H. Nitschmann, Proton-pumping cytochrome oxidase in the cytoplasmic membrane of *Anacystis nidulans*, in: G.C. Papageorgiou, L. Packer (Eds.), Photosynthetic Prokaryotes: Cell Differentiation and Function, Elsevier Biomedical Press, New York, 1983, pp. 147–162.
[218] G.A. Peschek, Proton pump coupled to cytochrome *c* oxidase in the cyanobacterium *Anacystis nidulans*, J. Bacteriol. 153 (1983) 539–542.
[219] G.A. Peschek, Characterization of the proton-translocating cytochrome *c* oxidase activity in the plasma membrane of intact *Anacystis nidulans* spheroplasts, Plant Physiol. 75 (1984) 968–973.
[220] H.W. van Verseveld, K. Krab, A.H. Stouthamer, Proton pump coupled to cytochrome *c* oxidase in *Paracoccus denitrificans*, Biochim. Biophys. Acta 635 (1981) 525–534.
[221] S. Arnold, B. Kadenbach, Cell respiration is controlled by ATP, an allosteric inhibitor of cytochrome-*c* oxidase, Eur. J. Biochem. 249 (1997) 350–354.
[222] D. Pils, G. Schmetterer, Characterization of three bioenergetically active respiratory terminal oxidases in the cyanobacterium *Synechocystis* sp. strain PCC6803, FEMS Microbiol. Lett. 203 (2001) 217–222.
[223] T. Volkmer, M. Ambill, M. Gendrullis, R. Dworah-Nkruma, D. Schneider, M. Rögner, Purification and characterization of cyanobacterial cytochrome b_6f and *bd* complexes, Book of abstracts #383, 13th Intern Congress of Photosynthesis, Montreal, Canada, 2004, p. 146.