Chapter 27

Respiration and Respiratory Complexes

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Summary

Respiration in facultative phototrophs is a flexible metabolic process that involves various electron donors and acceptors. A good example of such respiratory flexibility can be found in *Rhodobacter* species, most of them being equipped with genes that encode five distinct oxidases having different oxygen affinities. One of these, the cytochrome cbb_3 oxidase is prevalent at low oxygen tensions, and terminates a highly coupled electron transfer pathway which is formed by a 'core' of redox components, e.g., quinones, the cytochrome bc_1 complex and cytochrome *c*, in common with the photosynthetic apparatus. Thus, by modulating expression of different terminal oxido-reductases that lock onto a core electron transfer pathway, *Rhodobacter* species can survive in a range of oxic, micro-oxic, and anoxic environments either in the dark or in the light.

This chapter covers first the types and basic characteristics of the terminal oxidases in a few *Rhodobacter* species; then, respiratory substrates other than oxygen are examined. These substrates include orthodox anaerobic electron acceptors such as DMSO or TMAO but also arsenics as unconventional bioenergetics substrates. Finally, a synopsis of the data examining the functional interactions between photosynthetic and respiratory ETP is given along with a phylogenetic scenario suggesting that respiration is more ancient than both anoxygenic and oxygenic photosynthesis.

I. Aerobic Respiration

The term 'aerobic respiration' refers to the process of transferring high-energy electrons, derived from reduced organic or inorganic substrates, through a series of electron carriers to O_2. Organic and transition metal electron carriers are organized into multi-subunit integral membrane protein complexes in the bacterial cytoplasmic membrane, while NADH, water soluble or lipid anchored cytochromes *c*, and lipid soluble quinone transfer electrons between these complexes. Three of these multi-subunit enzymes, the proton pumping NADH dehydrogenase, the cytochrome bc_1 complex and the terminal oxidases are electrogenic, in that each of them has evolved one or more mechanisms to convert the energy released by electron transfer reactions into a voltage gradient across the cytoplasmic membrane. The voltage gradient is used by the F_1F_o ATP synthase to synthesize ATP from ADP and inorganic phosphate. In the longest known respiration chain, NADH, produced by NAD^+ reduction in cytosolic reactions, is oxidized by NADH dehydrogenase (Fig. 1). This enzyme transfers electrons to membrane-soluble ubiquinone, which diffuses into the bilayer. Reduced quinone is then oxidized by the cytochrome bc_1 complex that conveys electrons to cytochrome *c*. Finally, reduced cytochrome *c* is oxidized by a cytochrome *c* oxidase, which uses these electrons to reduce O_2 to H_2O. Reduced quinone (i.e., quinol) may also be oxidized by a quinol terminal oxidase, in a shorter chain that bypasses the cytochrome *c* pathway. Electrons may enter the respiratory system at various points, as NADH from cytosolic reactions, from reactions that reduce UQ (such as succinate dehydrogenase) or from periplasmic oxidation reactions that reduce various cytochromes *c*.

Rhodobacter (*Rba.*) *sphaeroides* and *Rba. capsulatus* are two of the most intensively studied prokaryotes in terms of their structural, functional and genetic features of aerobic respiration; as such they provide useful focal points for this discussion. As studies of the cytochrome bc_1 complex and the ATP synthase are presented elsewhere in this volume (Chapter 22, Berry et al.; Chapter 23, Kramer et al.; Chapter 24, Feniouk and Junge), they will not be discussed here.

A. Respiratory Complexes and Electron Transport Carriers

1. NADH Dehydrogenase (NADH:Q Oxidoreductase)

The aerobic respiration system of *Rba. sphaeroides* begins with the presence of two versions of the proton-pumping NADH dehydrogenase (termed NDH-1), as predicted by the genome (Joint Genome Institute Microbial Genomics, 2006, http://genome.jgi-psf.org/mic_home.html). Both predicted com-

Abbreviations: *C.* – *Chloroflexus*; C*c*O – cytochrome *c* oxidase; DMS – dimethylsulfide; DMSO – dimethylsulfoxide; *E.* – *Escherichia*; HiPIP – high-potential iron-sulfur protein; *Rba.* – *Rhodobacter;* RC – photochemical reaction center; *Rps.* – *Rhodopseudomonas; Rsb.* – *Roseobacter; Rvu.* – *Rhodovulum; T,* – *Thermus;* TMAO – trimethylamine-N-oxide; TMPD – N,N,N′,N′-tetramethyl-p-phenylenediamine; UQ – ubiquinone

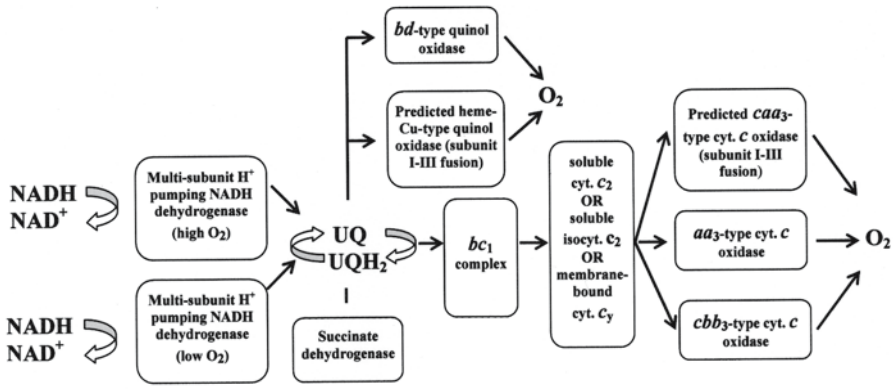

Fig. 1. The aerobic respiratory system of *Rhodobacter sphaeroides.* Note that expression of the genes for the two terminal oxidases containing fusions of subunits I and III is yet to be demonstrated.

plexes have high similarity to the core of the more elaborate mitochondrial enzyme (Other groups of bacteria also synthesize a single-subunit, non-proton pumping NADH dehydrogenase (NDH-2), and/or a sodium-translocating NADH dehydrogenase similar to NDH-1 but, thus far, these enzymes have not been found in the purple photosynthetic bacteria). NADH dehydrogenase is the largest of the bacterial electron transfer complexes, consisting of 14 protein subunits that contain a flavin cofactor and nine iron sulfur (Fe-S) centers (Friedrich and Scheide, 2000; Yagi et al., 2001). The characteristic L-shaped structure of the NADH dehydrogenase complex consists of a membrane-embedded arm plus an extramembrane domain that extends into the cytoplasm (Yagi and Matsuno-Yagi, 2003). A recent structure of the eight-subunit hydrophilic domain of *Thermus* (*T.*) *thermophilus* NADH dehydrogenase confirms that all of the redox centers are bound within the extramembrane domain (Sazanov and Hinchliffe, 2006). NADH is oxidized by flavin mononucleotide near the top of the extramembrane domain and the electrons are transferred to the site of quinone reduction, located at the interface of the extramembrane and membrane domains, near the cytoplasmic surface of the membrane. The structure of *T. thermophilus* NADH dehydrogenase emphasizes the well-recognized question of how electron transfer in the extramembrane domain drives proton pumping through the membrane domain of the complex. The *Rba. sphaeroides* 2.4.1 genome [as well as those of strains 2.4.3 (ATCC 17025) and 2.4.9 (ATCC 17029)] includes two *nuo* operons encoding this large complex, which are differentially regulated (Pappas et al., 2004). Under low oxygen conditions, the expression of one *nuo* operon is down-regulated while the other is enhanced. Interestingly, other members of the purple photosynthetic bacteria, including *Rba. capsulatus*, *Rhodospirillum rubrum* and *Rhodopseudomonas* (*Rps.*) *palustris* contain only one *nuo* operon, corresponding to the one that is upregulated in the presence of O_2 in *Rba. sphaeroides*.

Electrons from NADH dehydrogenase are transferred into the ubiquinone (UQ) pool in the membrane, from which they may enter the cytochrome bc_1 complex to flow via the 'cytochrome *c*' pathway to O_2, or the electrons may be shunted directly to a quinol-oxidizing terminal oxidase that reduces O_2. Here, the respiratory system is elaborated in two ways. First, the genome of *Rba. sphaeroides* encodes no fewer than five different terminal oxidases, and second, a variety of *c*-type cytochromes are present and capable of donating electrons to the cytochrome *c* oxidases. *Rba. sphaeroides* is more economical with regards to the cytochrome bc_1 complex; one operon encodes this complex, and the same enzyme participates in both the respiratory and photosynthetic electron transfer pathways. We will first discuss the types and basic characteristics of the terminal oxidases. Because the literature on terminal oxidases is vast, many of the citations refer the reader to reviews or recent articles where references to the original research may be found.

2. Cytochrome bd-type Terminal Oxidase

Two super-families of bacterial terminal oxidases are the cytochrome *bd*-type quinol oxidases, in which the site of O_2 reduction contains two heme groups that are buried within the largest subunit, and the heme-Cu oxidases that contain one five-coordinate

heme and a closely-associated copper atom at the similarly buried site of O_2 reduction (Garcia-Horsman et al., 1994). Cytochrome *bd*-type oxidases are common throughout aerobic eubacteria, but the most studied is that of *Escherichia* (*E.*) *coli* (Junemann, 1997). Of the two integral membrane subunits that form this complex, one, CydA, contains all of the redox centers as well as the proposed quinol binding site near the periplasmic surface (Dueweke and Gennis, 1991; Junemann, 1997; Mogi et al., 2006). A six-coordinate *b*-type heme mediates electron flow from quinol to the di-heme active site, composed of a *b*-type heme and an O_2-binding *d*-type heme. The *bd*-type oxidase is not a proton pump, although it is electrogenic because the protons required for O_2 reduction are taken up from the *n* (negative) side of the cytoplasmic membrane, through an undefined pathway, while the electrons from quinol are delivered from the *p* (positive) side of the membrane (Zhang et al., 2004) (In fact, all of the terminal oxidases are electrogenic in this way, whether or not they are also proton pumps). The architecture of the di-heme active site is argued to be responsible for the fact that the *bd*-type oxidase of *E. coli* has a high affinity for O_2, with a K_m of 3–8 nM (D'Mello et al., 1996; Borisov et al., 1994). A general characteristic of these enzymes is that a significantly higher concentration of cyanide is required to inhibit activity, as compared to heme-Cu oxidases (Junemann, 1997).

Compared to the *bd*-type oxidase of *E. coli*, relatively little is known about the *bd*-type oxidase of *Rba. sphaeroides*. Its transcript abundance is unaffected by ambient O_2 concentration (Pappas et al., 2004). It is present in the membranes of aerobically grown *Rba. sphaeroides* cells, but it accounts for much less of the total O_2 reduction activity than the cbb_3- or the aa_3-type oxidases (Hosler, unpublished). However, when the 'cytochrome *c* pathway' is inactivated in *Rba. sphaeroides,* thereby preventing electron flow to terminal oxidases that require reduced cytochrome *c*, the *bd*-type oxidase is capable of supporting aerobic growth (Yun et al., 1990; Cox et al., 2001).

Rba. sphaeroides makes no heme *d*, thus its *bd*-type oxidase contains a heme *bb* active site, as is the case in several other bacteria. The heme *bb*-subtype has not been purified and characterized, therefore it is not known to what extent, if any, the heme substitution affects O_2 affinity or oxidase activity. Apart from genomic information, the existence of a *bd*-type terminal oxidase in other members of the non-sulfur purple bacteria has been deduced from cyanide resistant O_2 reduction activity that is simultaneously resistant to inhibitors of the cytochrome bc_1 complex (Bonora et al., 1998).

3. Cytochrome cbb_3-type Terminal Oxidase

The cbb_3-type cytochrome *c* oxidases, members of the heme-Cu superfamily, are also widely distributed throughout the eubacteria. The catalytic core consists of three subunits, where the largest, CcoN, contains the heme b_3-Cu_B O_2 reduction site along with a low spin heme *b* that transfers electrons to the active site (Pitcher and Watmough, 2004). Electrons are transferred into CcoN by two other subunits, CcoO and CcoP, which consist of membrane anchoring helices and periplasmic domains containing *c*-type hemes. CcoO contains a single *c*-type heme, while CcoP contains two *c*-type hemes. Whether these two subunits function independently or in series to transfer electrons from cytochrome *c* to CcoN is not clear, although a homology model of the cbb_3-type cytochrome *c* oxidase (C*c*O) of *Rba. sphaeroides* suggests that the latter is more likely (Sharma et al., 2006). Based on the homology of CcoN and CcoO to the two subunits of NO reductase, CcoO is thought to be the immediate electron donor to the low spin heme of CcoN. Whether di-heme CcoP is absolutely required for the activity of all cbb_3-type C*c*Os is yet unclear. Inactivation of the gene for CcoP in *Bradyrhizobium japonicum* eliminates much but not all of the activity of the complex (Zufferey et al., 1996), while inactivation of its homolog in *Rba. capsulatus* eliminates all activity (Koch et al., 1998). In addition, the site(s) at which soluble cytochrome *c* or membrane-bound cytochrome c_y bind to donate electrons remains to be identified. A fourth subunit, CcoQ, is a small peptide with a single transmembrane helix that appears to stabilize the CcoNOP complex, possibly by minimizing proteolysis (Oh and Kaplan, 2002). The *ccoGHIS* operon encoding proteins required for the assembly of the cbb_3-type C*c*O is located adjacent to the *ccoNOQP* operon encoding this enzyme. The function of the *ccoGHIS* gene products is discussed in Chapter 21 (Sanders et al.).

Transcript abundance for cbb_3-type C*c*O of *Rba. sphaeroides* increases as ambient O_2 decreases (Pappas et al., 2004), although the cytoplasmic membranes of aerobically-grown *Rba. sphaeroides* cells still contain large amounts of the enzyme. In fact, the cbb_3-type C*c*O of *Rba. sphaeroides* appears to be present under all laboratory growth habits. The affinity of the

cbb$_3$-type C*c*O for O_2 has only been measured for the *Bradyrhizobium japonicum* enzyme, where it is only expressed under microaerobic or anaerobic conditions. Even though this oxidase contains a heme-Cu active site, a K_m of 7 nM O_2 was obtained (Preisig et al., 1996), essentially the same as for the *bd*-type oxidases (see above). Unlike the *bd*-type oxidases, the *cbb*$_3$-type C*c*Os pump protons from the *n* side to the *p* side of the cytoplasmic membrane, in addition to generating a transmembrane voltage gradient by consuming substrate protons from the *n* side. This proton pumping activity has been demonstrated in several species, including *Rba. sphaeroides* (Toledo-Cuevas et al., 1998).

Particularly intriguing is the role of the *cbb*$_3$-type C*c*O in gene regulation. Synthesis of the photosynthetic apparatus of *Rba. sphaeroides* normally requires anaerobic or microaerophilic growth conditions. However, when the genes for the *cbb*$_3$-type C*c*O are deleted from the cell, photosynthesis proteins accumulate during aerobic growth (Oh and Kaplan, 1999). Preventing electron flow to the *cbb*$_3$-type C*c*O by deleting genes directing the synthesis of the cytochrome bc_1 complex or cytochromes c_2 and c_y has the same effect (Daldal et al., 2001; Rios-Velazquez et al., 2003). The connection between the *cbb*$_3$-type C*c*O and the photosynthesis genes is thought to occur through the PrrA/PrrB (also called RegA/RegB in *Rba. capsulatus*) response regulator/histidine kinase system. Normally, the level of electron flow through the *cbb*$_3$-type C*c*O modulates the kinase/phosphatase activities of PrrB such that greater electron flow leads to greater repression of photosynthetic gene expression (Oh et al., 2004). The biochemistry that links electron flux through the *cbb*$_3$-type C*c*O to PrrB activity remains to be elucidated. The redox state of the quinone pool, which will vary with the rate of electron flow to O_2, affects the autophosphorylation activity of RegB of *Rba. capsulatus*, the homolog of PrrA in *Rba. sphaeroides* (Swem et al., 2006). However, recent experiments show that mutation of a single conserved histidine residue of in *Rba. sphaeroides* CcoN, not a heme ligand, breaks the sensor linkage in that electron flux through the mutant *cbb*$_3$-type C*c*O is the same as through the wild-type enzyme but the photosynthetic genes are not repressed under aerobic conditions (Oh, 2006). This argues against the involvement of the quinone, since the high electron transfer activity of the mutant oxidase should maintain the same redox poise of the quinone pool as wild-type *cbb*$_3$-type C*c*O.

4. Cytochrome aa$_3$-type Terminal Oxidase

The *aa*$_3$-type C*c*O of *Rba. sphaeroides* is the best characterized terminal oxidase of the purple photosynthetic bacteria, with three published crystal structures (Svensson-Ek et al., 2002; Qin et al., 2006) and numerous structure/function studies via site-directed mutagenesis (Richter and Ludwig, 2003; Branden et al., 2006a; Hosler et al., 2006). The twelve transmembrane helices of subunit I follow the architecture of other members of the heme-Cu oxidase family, binding the six-coordinate heme *a* center plus five-coordinate heme a_3 with a nearby copper (Cu_B), which function together as the O_2 reduction center. Subunit II contains two transmembrane helices that anchor a periplasmic domain containing the di-copper Cu_A center. Subunit III consists of seven transmembrane helices, bound to the face of subunit I opposite that of the helices of subunit II. Its function will be discussed below. A small subunit IV, of unknown function, contains a single transmembrane helix that lies against subunits I and III. Subunits I, II and III are closely related to the catalytic core of mitochondrial C*c*O, making this *Rba. sphaeroides* enzyme an appropriate experimental model for human C*c*O.

In the catalytic mechanism of the *aa*$_3$-type C*c*O, cytochrome *c* binds to subunit II and donates its electron to Cu_A (Wang et al., 1999). The electrons then flow to heme *a* in subunit I, and then to the heme a_3-Cu_B center (Hill, 1994). One or two substrate protons (those destined for water) are taken up from the *n* side of the membrane during the reduction of the active site (reviewed in Hosler et al., 2006). Once both heme a_3 and Cu_B are reduced, O_2 binds transiently to Cu_B and then to heme a_3 (Lemon et al., 1993). Current evidence indicates that O_2 is rapidly reduced by four electrons (Proshlyakov et al., 1998; Babcock, 1999; Morgan et al., 2001), two from the reduced heme a_3, one from reduced Cu_B, plus one from a covalently cross-linked histidine-tyrosine group in the active site (Yoshikawa et al., 1998) that forms a radical (Proshlyakov et al., 2000; Proshlyakov, 2004). The histidine of the cross-linked His-Tyr pair is one of the three ligands of Cu_B. With this concerted four-electron reduction of O_2, the O-O bond is broken and the first water is formed, which binds to Cu_B as hydroxide, while the other oxygen atom forms an oxoferryl of heme a_3 (a_3^{4+}=O^{2-}). The oxoferryl and the histidine-tyrosine radical persist in the active site until two more electrons are delivered by the cytochrome *c*-Cu_A-heme *a* pathway, and the

remaining substrate protons are delivered (Adelroth and Brzezinski, 2004). These electrons and protons 1) protonate the hydroxide on Cu_B, releasing water; 2) reduce the oxoferryl to heme a_3^{3+} plus a hydroxide, which binds to Cu_B; and 3) reduce the tyrosine radical. The aa_3-type C*c*O is an efficient proton pump, with one proton being transported through the protein, across the membrane, for every proton (or electron) that is delivered to O_2 (Wikström, 2004; Hosler et al., 2006).

The aa_3-type C*c*O proteins have a lower affinity for O_2 than the cbb_3- or *bd*-type oxidases, with reported K_m values of 0.4 to 5 μM (Poole et al., 1979; García-Horsman et al., 1991; Riistama et al., 2000; Pils and Schmetterer, 2001). Transcription of the genes for the aa_3-type C*c*O of *Rba. sphaeroides* requires high ambient O_2 (Pappas et al., 2004), although the mechanism of O_2 regulation is not yet clear. As an example of the requirement for high O_2, the aa_3-type C*c*O cannot be detected spectroscopically in cells taken from aerobic plate cultures whereas it is present once cells are grown in liquid culture with vigorous aeration (Hosler, unpublished). Since the biochemical functions of the cbb_3- and aa_3-type C*c*Os are the same (both are cytochrome *c* oxidases and both are proton pumps), but the cbb_3-type C*c*O is synthesized at both low and high ambient O_2, it becomes a question as to why the aa_3-type C*c*O is retained. Indeed, *Rba. capsulatus* synthesizes only the cbb_3-type enzyme. Increased transcription of the genes for the aa_3-type C*c*O of *Rba. sphaeroides* occurs in response to the addition of H_2O_2 (Zeller et al., 2005), raising the possibility that the aa_3-type C*c*O helps relieve oxidative stress.

5. A Putative caa_3-type Cytochrome c Oxidase and a Putative Quinol Oxidase

Genes for two additional terminal oxidases that have yet to be expressed under laboratory growth conditions are found in the genome of *Rba. sphaeroides*. The first of these is a putative caa_3-type C*c*O since the gene for its subunit II predicts that it should contain both Cu_A and a cytochrome *c* (Mackenzie et al., 2001). The presence of a caa_3-type enzyme in *Rba. sphaeroides* is remarkable, since these enzymes are generally found in Gram-positive bacteria that lack soluble, periplasmic, *c*-type cytochromes. Even more remarkable is the subunit I for this enzyme, predicted to be a fusion of a typical subunit I domain with an integral membrane protein that contains seven transmembrane helices. The predicted organization of this latter domain is similar to subunit III of the aa_3-type C*c*Os, although the amino acid sequence of this region has little homology to known subunit III sequences. Such gene fusions (both subunit II-cytochrome *c* and subunit I-subunit 'III') are found in Archaea that grow in extreme environments. The genes for a fifth potential oxidase of *Rba. sphaeroides* appear to encode a heme-Cu oxidase with the ability to oxidize quinol (Mouncey et al., 2000). The largest subunit of this enzyme also appears to be a fusion of usual genes for subunit I plus a subunit III-like integral membrane protein. The notion that this terminal oxidase accepts electrons from quinol is derived from the observation that its predicted subunit II is similar to those of other quinol oxidases in the heme-Cu family. The genes for these two oxidases do not appear to be expressed under conditions tested thus far (Mouncey et al., 2000), and attempts to express them from other promoters have not met with success. Both sets of genes are found in the genomes of the three strains of *Rba. sphaeroides* that have been sequenced to date, and the genes for the caa_3 form with fused subunits I and III are present in the genomes of *Rps. palustris* strains as well (Joint Genome Institute Microbial Genomics, 2006, http://genome.jgi-psf.org/mic_home.html). This suggests that members of the purple photosynthetic bacteria can perform aerobic respiration under some specialized, perhaps extreme, condition that has yet to be identified.

6. Cytochromes c and HiPIPs (High-Potential Iron-sulfur Proteins)

Rba. sphaeroides synthesizes several *c*-type cytochromes that mediate electron transfer between the cytochrome bc_1 complex and the various C*c*Os, including the soluble cytochrome c_2 (Meyer and Cusanovich, 1985), isocytochrome c_2 (Rott et al., 1993) and membrane-bound cytochrome c_y (Jenney and Daldal, 1993). All three of these proteins are efficient donors to both the cbb_3- and the aa_3-type C*c*Os (Hochkoeppler et al., 1995b; Daldal et al., 2001). The relative affinities of all three cytochromes for each C*c*O and the maximum rates of O_2 reduction that they can support are all similar (Drosou et al., 2002; Donohue and Hosler, unpublished). Therefore, if there is a preferred cytochrome *c* substrate for either oxidase in vivo it has yet to be identified. All three cytochromes *c* are present in aerobically grown cells. Transcript abundance for *Rba. sphaeroides*

cytochrome c_2 is increased in the absence of O_2, consistent with its role in photosynthetic electron transfer, but transcription of the genes for isocytochrome c_2 and cytochrome c_y are higher in the presence of O_2 (Pappas et al., 2004).

In the middle of the nineties, after almost three decades of studies on the fundamental role of the soluble cytochrome c_2 in both photosynthesis and respiration of purple phototrophs, it became clear that high-potential iron sulfur proteins (HiPIPs) are soluble electron carriers alternative to cytochrome(s) *c*, in most phototrophic species of the genera *Rhodoferax*, *Rhodocyclus*, *Rhodospirillum*, *Chromatium* and *Ectothiorhodospira* (Meyer and Donohue, 1995; Ciurli and Musiani, 2005). In *Rhodoferax fermentans*, a facultative phototroph lacking soluble cytochrome c_2 but expressing high amounts of HiPIP, light-induced respiration (also called LIOU, for Light-Induced Oxygen Uptake; Zannoni et al., 1998) is dependent on the concentration of the HiPIP ($E_0' = + 351$ mV) (Hochkoeppler et al., 1995a; Hochkoeppler et al., 1995c). Cells of the halophilic facultative phototroph *Rhodospirillum salinarum* (reclassified as *Rhodovibrio salexigens* by Imhoff et al., 1998) grown aerobically in the dark contain small amounts of cytochrome *c′* along with two isoforms of HiPIPs (Moschettini et al., 1999; Hochkoeppler et al., 1999). Since in isolated membrane fragments the HiPIP-iso1 was shown to greatly accelerate the aa_3-type C*c*O activity, it has been suggested that this isoform is indeed a functional component of *Rsp. salinarum* respiratory chain (Hochkoeppler et al., 1999).

B. Possible Reasons for Complex Respiratory Systems

While mitochondria contain a single route for electron transfer from NADH to O_2, purple photosynthetic bacteria such as *Rba. sphaeroides* contain numerous pathways. Several reasons for such complexity can be proposed, even though definitive evidence for these functions is not strong, and may be difficult to obtain by standard methods. The most obvious reason for multiple terminal oxidases is to allow the bacterium to adapt to differing ambient O_2 concentrations. Transcript abundance data from gene chip analyses are consistent with this notion, however the cbb_3-type C*c*O, with its higher affinity for O_2, is also present in large amounts along with the aa_3-type C*c*O in aerobically-grown cells of *Rba. sphaeroides*. In addition, the *bd*-type quinol oxidase, which may have the highest affinity for O_2, is expressed at both high and low ambient O_2. Determination of the amount of each oxidase present, and the relative flux of electrons through each, at differing O_2 concentrations, is required to fully understand how various respiratory systems respond to O_2.

The cbb_3- and *bd*-type oxidases almost certainly protect the photosynthetic apparatus from damage by O_2, by removing O_2 at the cytoplasmic membrane and keeping the environment of the intracytoplasmic membrane anaerobic. A possible related use of these oxidases is to poise the redox levels of the cytochrome *c* and the quinone pools for optimum cyclic electron transfer during photosynthesis. Since over-reduction of either pool may slow cyclic electron transfer, rendering photosynthesis less efficient for ATP production, either the *bd*- or the cbb_3-type oxidase may be used to shunt excess electrons to trace amounts of O_2 during photosynthesis.

Another obvious, but unproven, use for multiple respiratory pathways is to allow the cell to modulate its efficiency of ATP synthesis. For example, the transfer of two electrons from NADH to O_2 via the *bd*-type terminal oxidase should result in a total of six charge separation events (equivalent to six pumped protons), while the transfer of the same two electrons to O_2 via the cytochrome bc_1 complex and the C*c*Os should result in a total of ten charge separation events (ten pumped protons). Thus, the choice of pathway may lead to a 167% increase in the efficiency of ATP synthesis. Whether or not the cell actually utilizes more efficient pathways in response to energetic demand, or simply increases the rate and amount of less efficient pathways, requires more investigation. Shorter respiratory pathways may also help the cell regenerate NAD^+ under conditions where the ATP/ADP pool is already highly phosphorylated. Other reasons for the occurrence of specific oxidases have already been discussed (e.g., the regulatory function of the cbb_3-type C*c*O)

C. Structure-function Studies of the aa_3-type Cytochrome c Oxidase

The close similarity of the aa_3-type C*c*O of *Rba. sphaeroides* to the catalytic core of mitochondrial C*c*O makes it a useful experimental model. Structure-function analyses of *Rba. sphaeroides* C*c*O became possible with two key advances. The first was the development of a mutagenesis system (Shapleigh et al., 1992; Hosler et al., 1993), while the second

was the release of a high resolution crystal structure containing all four subunits, the redox centers, the Mg center between subunits I and II and six tightly-bound lipids (Svensson-Ek et al., 2002). A higher resolution structure has recently been obtained for subunits I and II (Qin et al., 2006). The following is a short discussion of a few of the salient findings from studies of the *Rba. sphaeroides* enzyme, although studies of other systems, particularly the aa_3-type C*c*O of *Paracoccus denitrificans*, have also contributed greatly to the current understanding of C*c*O function (Richter and Ludwig, 2003).

1. Proton Pathways and Proton Pumping.

Two proton pathways lead from the inner surface of C*c*O (the cytoplasmic or negative surface) toward the buried heme a_3-Cu_B center. These were identified in C*c*O structures with the help of knowledge obtained in previous mutagenesis studies (Hosler et al., 1993; Fetter et al., 1995; Mitchell et al., 1996). For the D pathway, protons enter via the surface residue Asp 132 of subunit I and are transferred ~26 Å through a series of hydrogen-bonded water molecules to Glu 286, located midway between hemes *a* and a_3 (Svensson-Ek et al., 2002). Substrate protons (those used to reduce O_2) flow from Glu 286 to the active site through a shorter series of waters. Because Glu 286 is buried, it has an unusually high pK_a (9.4), and it remains protonated due to rapid proton delivery from Asp 132 (Namslauer and Brzezinski, 2004). The K pathway for protons begins at Glu 101 of subunit II, on the inner surface but far from Asp 132 (Tomson et al., 2003). K pathway protons move ~ 26 Å via amino acid side chains and bound waters to Tyr 288, which is covalently linked to His 284, a Cu_B ligand.

The D and K pathways operate at different times during the catalytic cycle. The K pathway imports one (possibly two) proton as heme a_3 and Cu_B are being reduced prior to the binding of O_2 (Adelroth et al., 1998; Ruitenberg et al., 2000). This proton combines with a hydroxyl group on Cu_B, releasing the second water of the overall cycle, as discussed above in section I.D. Once O_2 binds, the D pathway brings in the remaining substrate protons (Adelroth and Brzezinski, 2004). The mechanism of how the active site accepts protons from one pathway or the other remains unsolved.

The D pathway provides all four of the protons that are pumped through C*c*O, across the membrane, as one O_2 is reduced to two waters (Branden et al., 2006a; Hosler et al., 2006). The pump is thought to be closely linked to the active site in subunit I, although the actual site and mechanism of pumping are not yet clear. The cleavage of the O-O bond by the concerted four-electron reduction of O_2 initiates a series of active site intermediates, all with the capability to draw protons into the protein (Branden et al., 2006a). Glu 286 of the D pathway appears to be a branch point from which protons are directed to O_2 reduction intermediates or to the proton pump. At four sequential conversions between active site intermediates, two protons are taken up from the negative surface of the membrane and one proton is ejected to the positive surface of the membrane. Of the two protons that are taken up from the negative surface, one is pumped while the other combines with an electron from the positive surface in the production of electrically neutral water.

Solving the pumping mechanism requires ingenuity. Mutagenesis of residues in or near the active site often inactivates the enzyme, but mutagenesis of more distant residues can provide important insights. For example, placing a carboxylate residue within the D pathway eliminates proton pumping while maintaining O_2 reduction (Pawate et al., 2002). It appears that electrostatic interactions raise the pK_a of Glu 286 to the point where it fails to protonate the pump, but Glu 286 can still be deprotonated by the O_2 reduction intermediates which have very high affinity for protons (Branden et al., 2006b). A key component of C*c*O is water, not all of which appears in the crystal structures. As the positions of waters become more defined, it is possible to deduce the sites of proton binding within the protein, e.g., those in water clusters, in hydrogen bonds and on amino acid side chains such as the ligands of Cu_B in the active site. This detail allows the formulation of more chemically precise pumping mechanisms such as one recently derived by first describing the positions of 'conserved' waters in subunit I by phylogenetic and structural analyses (Sharpe et al., 2005). Higher resolution structures increase the confidence of structural water assignments (Qin et al., 2006). In addition to mutagenesis, testing of proton transfer mechanisms is being pursued by a wide variety of computational methods (reviewed in Hosler et al., 2006).

Another mystery to be solved is the path that transfers protons from the site of pumping to the outer surface of C*c*O. A single proton exit pathway is not apparent in the C*c*O structures. An important finding in this search is that of *reverse* proton flow,

presumably through the normal exit pathway (Mills et al., 2002). Under conditions (such as a high transmembrane voltage gradient) that inhibit proton uptake from the inner surface, C*c*O maintains a slower flow of protons from the outer surface to the active site. An increasing collection of mutants that affect proton backflow should help map this pathway (Mills et al., 2005). Proton backflow may have an important protective effect for C*c*O (see below).

2. Protection from Oxidative Damage

Enzymes that reduce O_2 are prone to oxidative damage. As described above, once O_2 binds to reduced heme a_3, a four electron reduction rapidly converts diatomic O_2 to a bound oxoferryl ($a_3^{4+}=O^{2-}$) and a hydroxyl anion on Cu_B. The radical of the Tyr 288–His 284 group is also generated. The rapid reduction mechanism has the benefit that the one and two electron reduction intermediates, superoxide and H_2O_2, cannot be released into the cell. A disadvantage is that two potent oxidizing agents, the oxoferryl and the tyrosine radical, persist within the protein until further electrons and protons are delivered to the buried active site. Interaction of these oxidizing agents with the surrounding protein, if allowed to occur, is likely to be detrimental. For the aa_3-type C*c*O, a major part of the solution to this problem appears to be subunit III. Subunit III is as conserved as subunit I, but it contains no metal centers, and thus plays no direct role in electron transfer or O_2 reduction. Subunit III does bind two structural lipids within a deep cleft; one of the lipids lies against subunit I and comes within 15 Å of the active site (Svensson-Ek et al., 2002). Subunit III can be removed from C*c*O using strong non-ionic detergent. The resulting sub-complex is highly stable, has near normal O_2 reduction activity and pumps protons. However, with continued catalytic turnover subunit III-deficient C*c*O rapidly and irreversibly loses activity, or suicide inactivates (Bratton et al., 1999; Hosler, 2004). Suicide inactivation has been traced to events at the heme a_3-Cu_B center, and it is much more probable under conditions that extend the lifetime of the oxoferryl during the catalytic cycle, pointing toward this intermediate as the causative agent (Mills and Hosler, 2005), although the tyrosine radical might also be involved. Thus far, studies indicate that subunit III normally functions to prevent suicide inactivation in three ways. First, subunit III enhances proton uptake into the D pathway, which shortens the lifetime of the oxoferryl during the catalytic cycle (Gilderson et al., 2003; Adelroth and Hosler, 2006). Second, subunit III promotes the activity of the proton backflow pathway in subunit I (the apparent reverse of the exit pathway for pumped protons) (Mills et al., 2003). This helps to limit the lifetime of the oxoferryl under conditions where proton uptake from the inner surface of C*c*O is inhibited. Finally, normal binding of the structural lipids in the cleft of subunit III is required to prevent suicide inactivation (Varanasi et al., 2006). The lipids appear to function as structural modifiers, perhaps decreasing protein movements associated with proton pumping that would bring the oxoferryl close to an internal target.

3. Cytochrome c Oxidase Assembly

The post-translational assembly of mitochondrial C*c*O requires a large number of proteins specific for this process. Several of these proteins have homologs in *Rba. sphaeroides*. A key advantage of studying C*c*O assembly in the bacterium, as opposed to mitochondria, is that partially assembled C*c*O forms accumulate in the membrane, from which they can be isolated and characterized (Bratton et al., 2000). For example, the isolation of a C*c*O form containing all of the redox centers except for Cu_B provides the direct physical evidence necessary to conclude that the copper-binding protein Cox11p is absolutely required for the assembly of Cu_B of the aa_3-type C*c*O (and not for the assembly of Cu_A) in both *Rba. sphaeroides* and in mitochondria (Hiser et al., 2000). Another assembly protein, Surf1, is not absolutely required for C*c*O assembly but early events in the assembly of the complex in mitochondria are markedly affected by its absence, leading to a deficiency of C*c*O (Stiburek et al., 2005). Recent work in *Rba. sphaeroides* reveals that Surf1 is required for the efficient assembly of heme a_3 of the active site in subunit I, but not for the assembly of heme *a* in subunit I or Cu_A in subunit II (Smith et al., 2005). Further details of C*c*O assembly are discussed elsewhere in this volume (Chapter 21, Sanders et al.)

D. Structure-function Studies of the cbb_3-type Oxidase

Analysis of the cbb_3-type C*c*O has lagged behind that of the aa_3-type enzymes. Emerging studies are of high interest since the comparison of proton pumping C*c*Os with considerable variations in structure

reveals common features necessary for O_2 reduction and proton pumping. A crystal structure of a cbb_3-type C*c*O is not yet available, but homology models of CcoN (subunit I) of the *Rba. sphaeroides* and *Vibrio cholerae* enzymes have been constructed (Hemp et al., 2005; Sharma et al., 2006). A proton channel analogous to that of the K pathway of the aa_3-type C*c*Os leads from the inner surface of CcoN to a His-Tyr group at the heme b_3-Cu_B active site (Sharma et al., 2006). The His-Tyr group at the active site appears analogous to that present in the active site of the aa_3-type C*c*Os but the tyrosine is located on a different transmembrane helix (Hemp et al., 2005; Sharma et al., 2006). Alteration of the tyrosine to a phenylalanine eliminates activity and the existence of the His-Tyr cross-link has recently been confirmed in both the *Rba. sphaeroides* and *Vibrio cholerae* cbb_3-type C*c*Os (Hemp et al., 2006; Rauhamaki et al., 2006). Thus, a His-Tyr group at the active site must be an essential feature in the heme-Cu terminal oxidases since it is found throughout this wide group regardless of other structural differences. A major difference between the aa_3- and cbb_3-type oxidases is the lack of an analog of the D pathway in the latter group. Further studies of the cbb_3-type C*c*O may help reveal the reason for the existence of two proton uptake pathways (D and K) in the aa_3-type enzymes.

II. Respiration Utilizing Substrates other than Oxygen

Many members of the α-, β- and γ-subgroups, in particular the facultative phototrophs, are commonly considered to be the most versatile of all prokaryotes with respect to energy metabolism. Species such as *Rba. capsulatus* and *Rba. sphaeroides* may grow aerobically and photosynthetically using either organic or inorganic substrates but also anerobically (in darkness) with trimethylamine-N-oxide (TMAO) or dimethylsulfoxide (DMSO) as electron acceptors. Oddly, the question of whether anaerobic reduction of TMAO or DMSO is a true respiratory process, or a peculiar example of fermentative process requiring accessory oxidants to proceed, is still open to debate (McEwan et al., 1985; Zannoni, 1995). Some strains of the species *Rps. palustris*, *Roseobacter* (*Rsb.*) *denitrificans* and *Rba. sphaeroides* can reduce nitrate (NO_3^-) into dinitrogen (N_2) via nitrite (NO_2^-), and in some cases also nitric oxide (NO) and nitrous oxide (N_2O) (see Chapter 31, Shapleigh). In addition to widespread substrates such as O_2, NO_3^-, H_2 or organic acids and alcohols, a plethora of less well-known redox chemicals present in the environments are recruited for bioenergetic purposes. These redox chemicals comprise 'nasty' substances such as arsenics, chlorates and perchlorates or halogenated aromates (see also Chapter 29, Harwood). Admittedly, not all of these substrates have been shown to be used by photosynthetic members of the proteobacteria (i.e., the purple bacteria). We consider, however, that this is at least partly due to the lack of experimental data rather than the actual absence of phototrophic proteobacteria performing these reactions. For decades, studies of purple bacterial energy conservation have focused on the photosynthetic pathways, or at best on the oxygen-respiratory mechanisms operative under aerobic conditions (i.e., at relatively high oxygen tensions). Consequently, the respiratory systems working under low oxygen tensions in the dark (i.e., the microaerophilic lifestyles), are only about to be elucidated in several purple bacteria. At the same time, genomic data on the phototrophs are scarce, and the purple bacteria are certainly under-represented in genome sequencing projects, most probably due to their low priority under a biomedical perspective of the microbial world. These considerations led us to include in this chapter not only those 'exotic' or unconventional types of energy conservation that have actually been observed in purple bacteria, but also those which we think are very likely to exist in various members of this class of proteobacteria. The respiratory reactions involving inorganic nitrogen are considered to be 'common', and the reader is referred to recent reviews covering these topics (Richardson, 2000; Ferguson and Richardson, 2004) as well as to Chapter 31 (Shapleigh). On the other hand, although microbial DMSO and TMAO respiration has been reviewed recently (McEwan et al., 2004; McCrindle et al., 2005), for the sake of completeness a brief description of this important anaerobic process is included here.

A. Dimethylsulfoxide and Trimethylamine-N-oxide Respiration

Organic sulfur compounds originating from dimethylsulfoniopropionate are largely present in marine habitats. Dimethylsulfoniopropionate is converted to dimethylsulfide (DMS, volatile) and acrylic acid (Taylor and Kiene, 1989; Visscher et al., 1995), and then DMS is used as electron donor in bacterial

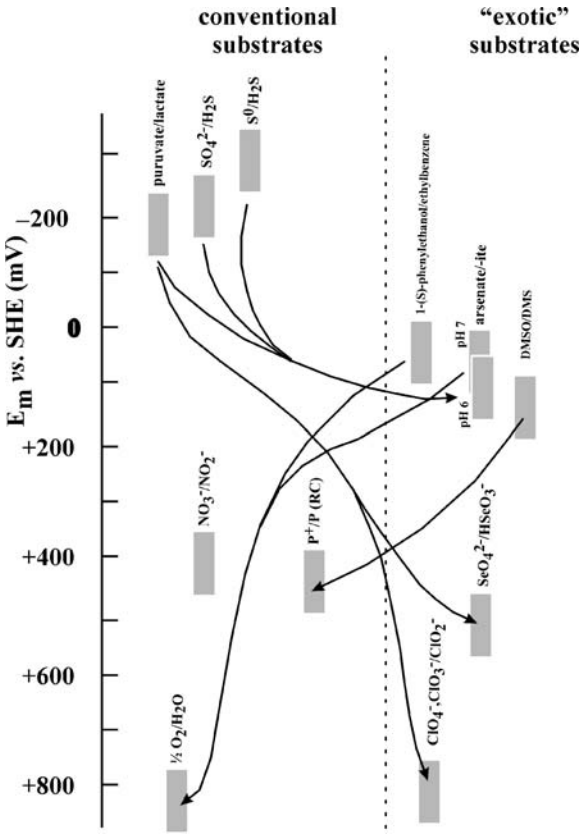

Fig. 2. Schematic representation of the electrochemical properties of electron donors and acceptors to the enzymes DMS dehydrogenase, ethylbenzene dehydrogenase, arsenite oxidase, arsenate reductase, chlorate and selenate reductase and the respective redox reactions. E_m values are given with respect to the standard hydrogen electrode (NHE) and the extent of the grey bars corresponds to the range of 10% to 90% reduction of the respective redox chemicals. The E_m value of the substrate to nitrate reductase is indicated for comparison.

photosynthesis (Visscher and Taylor, 1993). As a consequence, DMSO has been detected in seawater at relatively high amounts (Kelly and Smith, 1990), as it also returns in rainfall from the atmosphere where DMS is photochemically converted to DMSO.

DMSO can be used in a variety of nutritional modes by prokaryotes mainly under anaerobic conditions as exemplified by *Rhodobacter* species (Zannoni, 1995). In this latter bacterial group, DMSO reductase is a periplasmic enzyme and possesses a twin-arginine (Tat) secretory N-terminal signal peptide to direct its export (Shaw et al., 1996). In *Rba. capsulatus,* DMSO reductase is encoded by the *dorA* gene located in the same operon as the *dorC* gene, encoding a pentaheme *c*-type cytochrome of the NirT family (Shaw et al., 1999b). In *Rhodobacter* species the K_m of this enzyme for DMSO is in the micromolar range while its K_m for TMAO, another molecule acting as electron acceptor in bacterial respiration, is in the millimolar range (Johnson and Rajagopalan, 2001). The latter high K_m value for TMAO would suggest that this electron acceptor is physiologically non-relevant to most purple phototrophs. However, the marine aerobic photosynthetic species *Rsb. denitrificans* has been shown to reduce TMAO (Arata et al., 1992). On the other hand, the TMAO-reductase of *Rsb. denitrificans* is slightly different from that present in *Rhodobacter* species suggesting that its primary role might be in the TMAO respiration. Studies in *E. coli* show that the key feature that distinguishes TMAO reductase from DMSO reductase is that the former enzyme has a very low activity towards S-oxides (Iobbi-Nivol et al., 1996).

The E_0' of the DMSO/DMS couple is +160 mV while that of the TMAO/Trimethylamine (TMA) couple is +130 mV (Wood, 1981; Gon et al., 2001), indicating that both DMS and TMA are potential electron donors (and acceptors) in bacterial respiration and in photosynthetic metabolism. Indeed, the purple phototroph *Rhodovulum* (*Rvu.*) *sulfidophilum* strain SH1 was shown to be capable of photolithotrophic growth when DMS was used as the sole electron donor and DMSO accumulated as a product (Hanlon et al., 1994). DMS dehydrogenase has been purified from *Rvu. sulfidophilum,* and it has been shown to contain *bis*(molybdopterin guanine dinucleotide)Mo (McDevitt et al., 2002a). DMS dehydrogenase is coded by the *ddh* gene cluster, which comprises the *ddhA, B*, *C* and *D* genes. The *ddhA* gene encodes a polypeptide with highest sequence similarity to the molybdopterin-containing subunits of selenate reductase and ethylbenzene dehydrogenase, while *ddhB* contains cysteine-rich sequence motifs suggesting that it contains multiple iron-sulfur clusters (McDevitt et al., 2002b). DMS dehydrogenase is part of a larger family of enzymes which will be treated below. The *ddhD* gene product is deduced to be cytoplasmic and water soluble, and may act as a molecular chaperone specific for the assembly of DdhAB. The observation that DdhD is not a component of purified DMS dehydrogenase is consistent with this view. It is interesting that DdhD is related to SerD (selenate reductase) and NarJ (nitrate reductase). This suggests a common origin in line with the evolutionary relationship between these type II enzymes, according to an earlier classification of enzymes of the DMSO

reductase family (Trieber et al., 1996; Blasco et al., 2001). In contrast to the DMSO reductase mentioned above, the *ddhC* product appears to be secreted *via* the Sec-mediated pathway of secretion, as an apoprotein into the periplasm, where it folds and incorporates its heme *b* prosthetic group. There are very few examples of periplasmic *b*-type cytochromes; the majority of cytochromes in that compartment are of *c*-type, with covalently attached heme (Thony-Meyer, 1997). How the DdhAB complex and DdhC are assembled together in the periplasm to form the mature DMS dehydrogenase is unknown.

1. Organization of Dimethylsulfoxide - Trimethylamine-N-oxide Respiratory Chains

Analysis of the organization of a variety of anaerobic respiratory electron transport chains has revealed that they use QH_2 dehydrogenases bypassing the cytochrome bc_1 complex containing pathway (Richardson, 2000). The NirT family of QH_2 nitrite reductases (Jungst et al., 1991) is mainly formed by tetraheme *c*-type cytochromes that are membrane bound but facing the periplasm. Thus, they are ideally located to transfer electrons from QH_2 to periplasmic terminal reductases. The *c*-type multiheme cytochrome involved in DMSO reduction by *Rba. capsulatus* is DorC encoded by the *dorC* gene (Shaw et al., 1999a). It is a pentaheme protein composed of a tetraheme domain similar to other proteins of NirT type, and a C-terminal domain that contains an additional *c*-type cytochrome. The redox potentials of the heme centers of the purified DorC have been shown to range from –34 to –276 mV (Shaw et al., 1999a), with the highest potential being assumed to correspond to the monoheme that acts as electron donor to DMSO reductase.

TorC is a pentaheme protein, which is composed of a tetraheme domain with strong sequence similarity to DorC (Gon et al., 2001). The redox potentials of the hemes in the tetraheme domain are below 0 mV while that of the monomeric *c*-type heme is about +120 mV. These thermodynamic features seem to fit with a model in which the tetraheme is involved in menaquinol (MQH_2) oxidation while the monoheme transfers electrons to the TMAO reductase.

Cytochrome c_2 is essential for electron transfer from DMS dehydrogenase to the reaction center (RC) during phototrophic growth of *Rvu. sulfidophilum* (McDevitt et al., 2002). Although DMS dehydrogenase does not appear to be essential for growth under aerobic conditions with DMS as the sole energy and carbon source, accumulation of DMSO indicates that DMS is oxidized during this growth mode (Hanlon et al., 1994). Under these conditions, DMS dehydrogenase is expected to pass electrons to an energy conserving C*c*O via a soluble cytochrome c_2. Thus, DMS respiration would contribute to the energy metabolism of the cell even during oxidation of carbon substrates by the cell (myxotrophy).

Recent analyses have shown that at least three sub-families exist within the DMSO reductase family (McEwan et al., 2002; for a phylogenetic grouping, see Fig. 4). Type I enzymes such as periplasmic nitrate reductase (Nap), formate dehydrogenases and assimilatory nitrate reductase (Nas) contain a [4Fe-4S] cluster at the N-terminus of the Mo-containing α-subunit, and have a cysteine or selenocysteine side chain as a ligand to the Mo atom. An exception to this rule is the arsenite oxidase (see section E.1. of this chapter) where the N-terminal iron-sulfur cluster is also of the cubane type but there appears to be no Mo-ligand amino acid side chain. Type II enzymes such as respiratory nitrate reductases (Nar), selenate reductases (Ser) and DMS dehydrogenases (Ddh) possess an N-terminal His/Cys motif that is involved in iron-sulfur cluster formation (Bertero et al., 2003; Jormakka et al., 2004). Type III enzymes exemplified by *Rhodobacter* DMSO reductase and TMAO reductase lack a cysteine rich motif at their N-termini, and are not associated with a β-subunit that contains multiple [Fe-S] clusters. The ligand to the Mo atom in enzymes of this sub-family is a serine residue. Additional structural and molecular details on DMSO reductases in *Rhodobacter* sp. can be found in McEwan et al. (2004).

B. Arsenics as Bioenergetic Substrates

1. Arsenite Oxidation

Although considered the mother of poisons, arsenics are rather widespread components of our environment. Both aquatic habitats and soil contain significant amounts of arsenics (0.1 to more than 1000 ppm in soil, 50 to 400 ppm in atmospheric dust, 2.6 ppb in sea water; Mukhopadhyay et al., 2002). In aquatic environments, which are not at extreme pH values, the biorelevant forms of arsenics are their oxyanions $H_3AsO_3/H_2AsO_3^-$ (reduced form: arsenite) and $H_2AsO_4^-/HAsO_4^{2-}$ (oxidized form: arsenate). Genome surveys provided evidence for the presence of the

enzyme arsenite oxidase in numerous bacterial and archaeal species (Lebrun et al., 2003; Lebrun et al., 2006). Although arsenite oxidation often appears as a simple detoxification process, its implication in bioenergetics has been demonstrated in a range of non-photosynthetic α- and γ-proteobacteria (Oremland and Stolz, 2005), as well as in the phototroph *Chloroflexus aurantiacus* (Duval and Schoepp, in preparation). Among the photosynthetic organisms possessing arsenite oxidase genes are, in addition to *Chloroflexus*, the green sulfur bacteria *Chlorobium phaeobacteroides* and *Chlorobium limicola* (see also Table 3 of Chapter 33, Borsetti et al.). It has been speculated that in *Chloroflexus* electrons originating from the oxidation of arsenite might enter the photosynthetic electron chains (Zannoni and Ingledew, 1985; Lebrun et al., 2003), and subsequent experiments have shown that expression of the enzyme is not upregulated from its constitutive basal level when the bacterium is grown anaerobically in the light and in the presence of arsenite. However, *Chloroflexus* appears to be able to use arsenite as an electron donor under microaerophilic photosynthetic conditions (Duval and Schoepp, unpublished) like *Rhizobium* sp. str. NT-26 or *Hydrogenophaga* sp. str. NT-14 (Santini et al., 2000; van den Hoven and Santini, 2004). The identification of the oxidases and electron carriers involved in these electron transfer chains are under study. In the case of the strains NT-26 and NT-14, a *c*-type cytochrome seems to be the electron acceptor of arsenite oxidases (van den Hoven and Santini, 2004; Santini et al., 2007). Implication of a cytochrome *c* oxidase has therefore been suggested. A unique case of a bacterium using arsenite as donor, and nitrate (instead of O_2) as terminal electron acceptor has been reported for *Ectothiorhodospira* strain MLHE-1 (Oremland et al., 2002). Since green sulfur bacteria are, in contrast to the facultatively photosynthetic *Chloroflexus*, considered to be obligate phototrophs, arsenite oxidase may in these species indeed donate electrons to the (RCI-type) photosynthetic reaction centers. A more in-depth understanding of the role of arsenite oxidation in green sulfur bacteria and possibly in purple bacteria has to await further studies.

The actual cellular localization of the enzyme arsenite oxidase is still under debate. Three previously purified enzymes (i.e., those of *Alcaligenes faecalis*, *Rhizobium* sp. strain NT-26 and strain NT-14; Anderson et al., 1992; Santini and van den Hoven, 2004; van den Hoven and Santini, 2004) are soluble. By contrast, nearly all arsenite oxidase activity is found in the spheroplast fractions obtained from *Alcaligenes faecalis* and *Herminiimonas arsenicoxydans* (Anderson et al., 1992; Muller et al., 2003), and in the membrane fragments (resulting from French press treatment) of *C. aurantiacus* (Lebrun et al., 2003; Duval and Schoepp, unpublished). The basic building blocks of arsenite oxidases are a small subunit, which is a member of the Rieske [2Fe-2S] protein family (Lebrun et al., 2003; Lebrun et al., 2006) (rendering membrane-attachment likely), and a molybdopterin-containing catalytic subunit belonging to the DMSO-reductase superfamily. A more detailed discussion of the subunit composition will be given below. The structure of the soluble form of the enzyme has been determined (Ellis et al., 2001).

2. Arsenate Respiration

In more oxidizing environments, i.e., where the oxidized form of arsenic, arsenate, is predominant, it can be used as terminal electron acceptor with acetate, pyruvate or lactate as an electron donor. This mechanism has been shown to operate in several γ–, δ- and ε-proteobacteria, as well as in a few members of the low GC Gram-positive bacteria, of the Deinococci and of Crenarchaeota (Oremland and Stolz, 2003). Therefore arsenate respiration currently appears much less widespread than arsenite oxidation.

The basic structure of the arsenate reductase consists of a molybdopterin containing catalytic subunit belonging to the DMSO-reductase superfamily, and a small Fe-S subunit containing four cubane centers, products of the *arrA* and *arrB* genes, respectively. As suggested by the results obtained on one of the two enzymes purified (Krafft and Macy, 1998), and by the majority of the identified sequences (Stolz et al., 2006; Duval and Schoepp, unpublished), such a two-subunit respiratory arsenate reductase would be soluble. The process by which these Arr enzymes receive electrons from the quinol pool therefore remains to be established. The Arr enzyme from *Chrysiogenes arsenatis* is proposed to be periplasmic, thus how it contributes to the formation of a proton gradient is enigmatic. The only presently known example of a membrane-bound enzyme is that from *Bacillus selenitireducens* (Afkar et al., 2003). However, in several *arr* operons detected in genome surveys a third gene named *arrC*, homologous to the *psrC* gene of polysulfide reductase, is present immediately upstream of *arrA* (see below Fig. 4) (Stolz et al., 2006). Since PsrC is the membrane anchor subunit of polysulfide

reductase (Krafft et al., 1992), the presence of this gene in *arr* operons is in favor of a membrane association of respiratory arsenate reductases.

C. Selenate Respiration

The microbial reduction of selenate mediated by bioenergetic electron transport in α-, β-, γ-, ε- proteobacteria and Gram-positive bacteria (Oremland et al., 1994; Switzer Blum et al., 1998), and in particular in their photosynthetic representatives (such as *Rba. sphaeroides*) was reported 15 years ago (Moore and Kaplan, 1992; Chapter 33, Borsetti et al.). In some of these species, selenate reduction was subsequently shown (Sabaty et al., 2001) to be a side-reaction of a more common anaerobic energy conserving pathway, i.e., denitrification, and more specifically the respiration of nitrate performed by enzymes of the nitrate reductase family (Nap and Nar) (Avazéri et al., 1997; Sabaty et al., 2001; Watts et al., 2005). More recently, the existence of an enzyme complex truly dedicated to selenate reduction was demonstrated in *Thauera selenatis* (Schröder et al., 1997)*, Sulfurospirillum barnesii* (Stolz and Oremland, 1999) and *Enterobacter cloacae* SLD1a-1 (Watts et al., 2003).

Selenate reductase, just as arsenite oxidase and respiratory arsenate reductase, belongs to the large superfamily of molybdopterin containing enzymes, and consists of, in addition to the large catalytic subunit harboring the molybdopterin moiety, a tetracubane Fe-S protein and a peculiar *b*-type cytochrome. A more comprehensive description of the structural features of this enzyme will be given below. Cellular localization of this enzyme seems to vary, since that from *Thauera selenatis* was reported to be soluble and in the periplasm, whereas that from *Enterobacter cloacae* has been demonstrated as being membrane-bound and facing the periplasm. Thus, as evoked for arsenate reductase above, how these enzymes are connected to the quinol pool, and how they generate proton gradient remain open questions.

In recent years, selenate reductase has turned out to be a representative of a well defined and quite conserved superfamily of enzymes, the members of which handle extremely divergent substrates that all function in energy conserving photosynthetic and respiratory chains.

D. The Selenate Reductase-type Class of Enzymes

At the time being, five distinct enzymes belonging to this class are known. These are selenate reductase (Schröder et al., 1997), chlorate reductase (Danielsson Thorell et al., 2003), DMS dehydrogenase (McDevitt et al., 2002), ethylbenzene dehydrogenase (Kniemeyer and Heider, 2001) and perchlorate reductase (Bender et al., 2005). All these enzymes share the same subunit architecture (perchlorate reductase being slightly divergent, see Fig. 3) with a catalytic molybdopterin subunit, a tetracubane iron sulfur protein and a *b*-type cytochrome, and are located in the periplasm of the organisms producing them.

DMS-dehydrogenase oxidizes dimethylsulfide in the purple non-sulfur bacterium *Rvu. sulfidophilum* (McDevitt et al., 2002), and injects the resulting reducing equivalents into a soluble cytochrome *c* which in turn serves as a reductant to the photooxidized special pair of the RCII-type reaction centre.

Chlorate and perchlorate reductase, as well as ethylbenzene dehydrogenase, have so far only been detected in non-photosynthetic proteobacteria. The three dimensional structure of ethylbenzene dehydrogenase from *Aromatoleum aromaticum* strain EbN1 has recently been solved (Kloer et al., 2006), providing an excellent structural model for the whole class of enzymes, considering their conserved subunit composition and the sequence similarity of the individual subunits.

E. Non-conventional Substrates but Conventional Bioenergetics

Although the above detailed substrates may appear strange, as they are mostly toxic, the energetics involved in the respective electron transport chains are nothing abnormal. Selenate and in particular chlorate are, due to their very high redox potentials at pH 7 (Fig. 2), ideal electron acceptors providing enormous throughput driving forces which, in the case of chlorate, comes close to those attainable by oxygen reduction. Arsenite is a reasonably good electron donor to aerobic respiration provided that it is present in sufficiently high concentrations in the environment, which is the case in most hydrothermal habitats. Consequently, it is not surprising that *C. aurantiacus* should be able to use this arsenic oxyanion for bioenergetic means. Finally, DMS is present in many marine habitats and thus lends itself as an

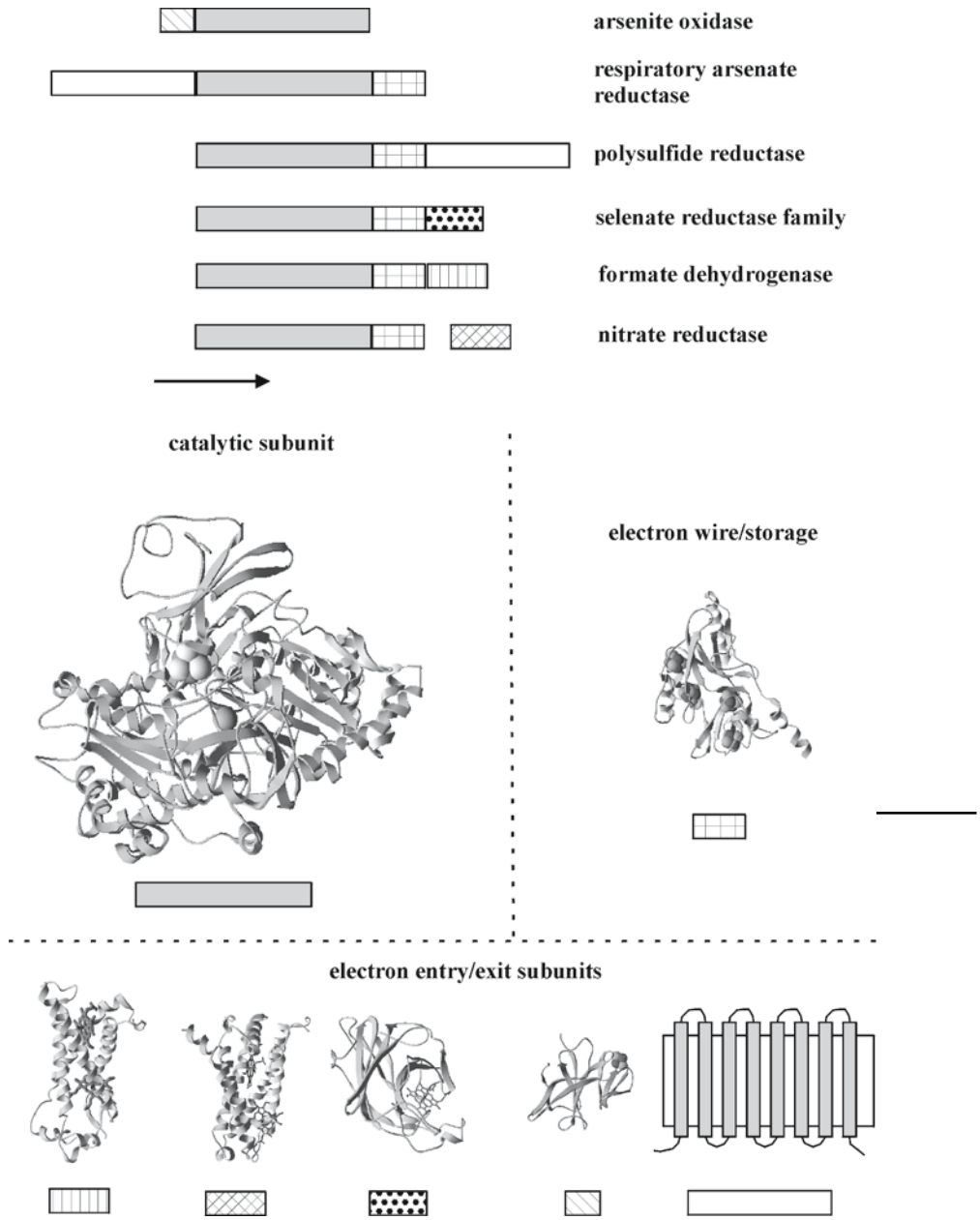

Fig. 3. Construction kit arrangement of the molybdoenzymes arsenite oxidase, arsenate reductase, polysulfide reductase, selenate reductase superfamily (including DMS dehydrogenase, ethylbenzene dehydrogenase and chlorate reductase) as well as formate dehydrogenase and respiratory nitrate reductase. The top part of the figure gives a schematic representation of the operon structure of the various complexes, whereas the bottom part shows the overall structures of the redox subunits involved using known 3-D structures of subunits from the set of enzymes. The structure of the membrane subunit of polysulfide reductase and respiratory arsenate reductase is not known so far and only a schematic version is represented.

alternative source of reducing equivalents in marine phototrophs. The only bioenergetic pathway that may be difficult to rationalize in terms of its energetics is arsenate respiration. Only a small $\Delta G_0'$ can be harvested from the electron transfer from pyruvate to arsenate (Fig. 2), so it is likely that the details of arsenate respiration are not yet well understood.

F. The Enzymes: Variations on a Theme

The enzyme complexes catalyzing the oxidoreduction reactions of these exotic substrates all use a molybdopterin protein belonging to the DMSO reductase superfamily as a catalytic subunit. In addition to this 'large' catalytic subunit, a small subunit, frequently a cytochrome, serves as entry or exit point for reducing equivalents. In between these two, a tetracubane iron sulfur protein plays the role of an electron wire, or an electron storage device, in most cases. The enzymes described above, together with the more common formate dehydrogenases, nitrate reductases and DMSO/TMAO reductases, provide examples of the modular composition observed in many bioenergetic enzymes (Baymann et al., 2003). Fig. 3 schematically summarizes the subunit composition of the respective complexes.

In this context, it may seem surprising that the subunit composition of the arsenite oxidizing and the arsenate reducing enzymes differ so substantially. Rather than mutating one enzyme, e.g., the oxidase, in specific places to modify electron transfer properties and thus reverse catalytic directionality, Nature apparently has chosen to invent a new enzyme. Instead of a Rieske protein, a tetracubane iron sulfur protein and most likely a large transmembrane subunit are present in arsenate reductase. Even the catalytic molybdopterin subunits of both enzymes show rather low sequence similarities. The Mo subunits of arsenate reductases are, in fact, closer to true DMSO reductases than to those of the arsenite oxidases.

The phylogenetic tree built from the sequences of diverse classes of enzymes using the molybdopterin subunit suggests a rationalization for the dissimilarity of arsenite oxidase and arsenate reductase. The tree in Fig. 4 shows all arsenite oxidases clustering together on a tight clade within the tree. The arsenite oxidase subtree itself is split into an archaeal and a bacterial cluster. This suggests that the evolutionary origin of arsenite oxidase pre-dates the divergence of Bacteria and Archaea more than 3 billion years ago (Lebrun et al., 2003). This early origin of arsenite oxidase is corroborated by the position of its Rieske subunit on a tree encompassing arsenite oxidases and Rieske/Cytochrome *b* complexes (Lebrun et al., 2006). The respective phylogenies of the Mo and the Rieske subunits quite clearly demonstrate that the enzyme arose at a time when the oxidation state of the environment of the Earth was relatively low. Under these conditions virtually no arsenate was present, hence there was no driving force to develop arsenate respiration.

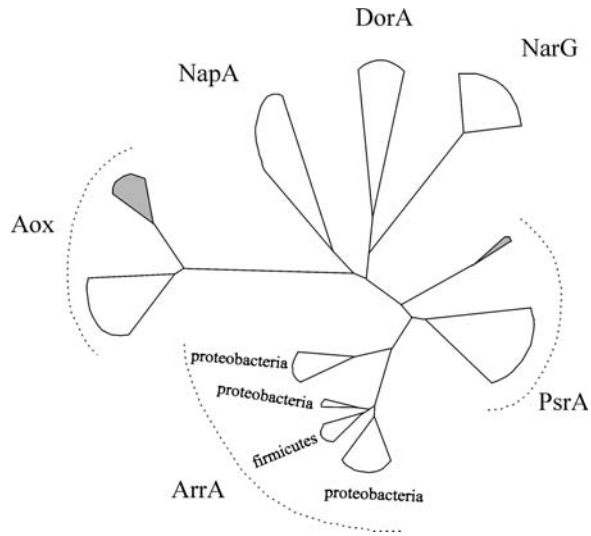

Fig. 4. Schematic representation of the phylogeny of the molybdopterin-containing catalytic subunits observed in arsenite oxidase (Aox), respiratory arsenate reductase (Arr), polysulfide reductase (Psr), membrane-bound nitrate reductase (Nar), periplasmic nitrate reductase (Nap) and DMSO reductase (Dor). Archaeal radiations are shown in grey shading. Phylogenetic trees were reconstructed based on the neighbor joining algorithm of Saitou and Nei (1987) using Clustal X and allowing for multiple substitutions.

The branch of the arsenate reductases, by contrast, shows all properties characteristic of an enzyme derived from another one at a later stage in evolution. Rather than from the stem of the entire tree, the reductase clade diverges from the subtree of the polysulfide reductases and does not feature an Archaea/Bacteria cleavage. Further, the branches of the arsenate reductases are quite mixed up as compared to the phylogenetic tree of their parent species, arguing for a distribution via lateral gene transfer. The comparable subunit composition of arsenate and polysulfide reductases (apart from a reshuffling of the operon structure, see Fig. 3) corroborates the scenario suggested by the phylogeny of the Mo subunits. The gradual advent of an oxidized environment brought about by oxygenic photosynthesis, and the concommitant accumulation of oxidized arsenic oxyanions therefore allowed for arsenate respiration to become energetically profitable and induced the evolution of a polysulfide reductase into an arsenate reductase (Duval and Schoepp, in preparation). The energy conserving processes based on arsenic oxyanions thus provide a textbook example of the intertwined

evolutionary history of bioenergetics and of the geochemical environment of the ancient Earth.

III. Respiration versus Photosynthesis: Which One Came First?

The concept that the early Earth's atmosphere was basically devoid of oxygen, with values <<1% of present atmospheric levels, is relatively widely accepted (Holland, 1994). However, reinterpretation of geological data, including the analysis of paleosols as well as iron buried in archaean sediments, has led to the proposal that significant amounts of oxygen would have been present in the primordial atmosphere (Towe, 1990, 1994, 1996; Ohmoto 1996, 1997). According to these studies, the amount of oxygen in the early atmosphere would have been at least 1 to 2% of the present values (Towe 1996; Ohmoto 1997). Accepting this latter proposal, it is evident that geological events, such as submarine hydrothermal vents, have contributed to the production of a wide range of reduced inorganic molecules such as H_2S, H_2, CO and NH_4^+, able to sustain a variety of aerobic and anaerobic respiratory processes (Madigan et al., 1997; Reysenbach and Shock, 2002). However, ATP-producing mechanisms using electron transfer to generate a proton gradient require both a well-formed membrane and the presence of an ATP synthase. Due to a parallel need for these two complex biological systems, respiration probably originated later than fermentation. Conversely, the order of appearance of chlorophyll-based photosynthesis and respiration seems to favor the latter one. Until recently, it was commonly assumed that, after fermentation, some type of primitive photosynthesis evolved in the most ancient cells (Cavalier-Smith, 2001). This assumption was based on 3500 Mya fossils taken as tracers of cyanobacteria (Schopf et al., 2002). However, these fossils have recently been reinterpreted as artifacts that arose from amorphous graphite (Brasier et al., 2002). Additionally, molecular phylogenesis and comparative analysis of key enzymes involved in photosynthesis and respiration support the view of a late emergence of photosynthesis relative to respiratory pathways (Castresana, 2001). In this context, there are five lineages within Bacteria with some type of bacteriochlorophyll-based photosystem. Purple bacteria (e.g., *Rhodobacter*, *Rhodopseudomonas*) and green non-sulfur bacteria (*Chloroflexus*) use a non-oxygen evolving type-II photosystem. Green sulfur bacteria (*Chlorobium*) and heliobacteria (*Heliobacterium*) use a type-I Photosystem. Finally, cyanobacteria use both Photosystems I and II for the more complex oxygenic photosynthesis. Since these five groups are not phylogenetically related, it is likely that the evolutionary pathways that gave rise to the different photosynthetic organisms are not as straightforward as it appears from the literature (Blankenship and Hartman, 1998; Baymann et al., 2001; Xiong and Bauer, 2002). Indeed, the close proximity of some non-oxygenic phototrophs to other non-phototrophs indicates that non-oxygenic photosynthesis has been lost at different stages in some evolutionary lines. In contrast, all known cyanobacteria perform oxygenic photosynthesis, and no other prokaryote outside this group has a similar metabolic option. This continuous maintenance of the same bioenergetic mechanism in such a large prokaryotic group, and for such a long time-scale period (Mya), is unique in the prokaryotic world. Though most textbooks still assume that oxygenic photosynthesis arose very early during the evolution of life, it is obvious from phylogenetic studies and biochemical considerations that oxygenic photosynthesis arose quite late, after the divergence of several other phototrophic bacterial groups (Brochier and Philippe, 2002). Thus photosynthesis, and particularly oxygenic photosynthesis, is not an ancient metabolic pathway. The presence of respiration before cyanobacterial type photosynthesis is consistent with the high affinity of the some terminal oxidases for oxygen, e.g., the cbb_3-type cytochrome *c* oxidases (see Section I.A.3) are functional at O_2 concentrations as low as 10^{-9} M (Preisig et al., 1996). Additionally, other bacterial populations living in niches totally devoid of oxygen would have used electron acceptors alternative to oxygen to sustain anaerobic respiration. These considerations, taken together, are sufficient to conclude that oxygenic photosynthesis appeared after respiration, and that it arose in Bacteria after their divergence from Archaea (Nitschke et al., 1997; Olson, 1999).

IV. Respiration and Photosynthesis are Intermingled

Continuous illumination of *Rhodobacter* cells partially inhibits both respiratory and denitrification activities, this latter process being present only under anaerobic conditions (Sabaty et al., 1993). Two non-mutually exclusive mechanisms were proposed to

explain the way respiration and photosynthesis might interact. First, the membrane potential generated by photosynthesis may affect the activity of electrogenic respiratory complexes in the membrane (Rugolo and Zannoni, 1983; Cotton et al., 1983). Second, there may be direct interaction between redox carriers common to both photosynthetic and respiratory chains (Zannoni et al., 1978).

What are the results that support the above proposals? The carotenoid-band shift, a specific indicator of the membrane potential present across the plasma membrane, is identical when induced either by photosynthesis or respiration (Wraight et al., 1978). This indicates that the $\Delta\mu H^+$ is delocalized over the entire cell membrane. This finding is supported by the fact that uncouplers prevent the inhibition of the respiratory activity by light (Rugolo and Zannoni, 1983; Richaud et al., 1986). The inhibition of respiration is essentially at the level of complex I, which is linked to light-dependent reversal of the electron flow from the Q pool (Cotton et al., 1983). Indeed, no back pressure of photosynthesis on respiration is seen at the level of the cytochrome bc_1 complex or C*c*Os, because continuous illumination has no effect on tetramethyl-p-phenylenediamine (TMPD)-dependent oxygen reduction (Sabaty et al., 1993).

A direct interaction between the diverse redox complexes has been shown by several approaches. Induction of a quinol oxidase activity by light with exogenous electron donors such as reduced horse heart cytochrome *c* or TMPD is observed with both membrane vesicles and hybrid-membrane fragments generated from electron transport mutants of *Rba. capsulatus* (Zannoni et al., 1978, 1986). This phenomenon might explain why under anaerobic conditions, and in the presence of reduced carbon sources such as succinate or butyrate, photosynthesis is partially inhibited by the reduction of the Q pool (McEwan et al., 1985). Under these strong reducing conditions continuous illumination can reactivate most of the RCs. This phenomenon might result from a fraction of functional RCs that are able to generate a membrane potential sufficient to reoxidize the Q pool by reversing the electron flow from quinol to NAD^+ (Herter et al., 1998). Reoxidation of the Q pool is also caused by oxygenation of the bacterial suspension, or by the addition of NO_3^- or DMSO (McEwan et al., 1985). This type of interaction is particularly important for aerobic photosynthetic bacteria such as *Roseobacter*, since under anaerobic conditions the primary quinone acceptor, Q_A, is totally reduced and no photochemistry occurs (Takamiya et al., 1987; Candela et al., 2001). Surprisingly, it has been shown that membranes from the facultative phototroph *Rba. capsulatus*, when subjected to continuous illumination, can generate ATP only under oxic conditions. Alternatively, light-induced phosphorylation can be measured under anoxic conditions, and at suitable redox poise, which is close to the $E_{m7.0}$ of the Q pool (~ +90 mV) (Candela et al., 2001). Whatever the molecular mechanism, it is apparent that the photosynthetic and respiratory chains must interact to favor optimal phosphorylation conditions. Another demonstration of this direct interaction has been provided by Richaud and coworkers (1986) using membrane fragments of *Rba. sphaeroides.* After a short flash of light, one electron is diverted from the respiratory chain to the photooxidized RC at the level of cytochrome c_2. Since two electrons are required to generate a QH_2 molecule at the secondary electron acceptor level, Q_B (*gating mechanism*; Verméglio, 1977), stimulation of respiration is seen after an even number of flashes of light.

Photosynthetic activity inhibits every step of the reduction of nitrate into dinitrogen (Sabaty et al., 1993). This inhibitory effect is not prevented by uncouplers, and therefore does not need the formation of a $\Delta\mu H^+$. It is postulated that this inhibition is due to the diversion of electrons from the denitrification chain to the photosynthetic cycle at the level of the cytochrome bc_1 complex and of soluble cytochrome c_2, components shared by the two pathways (Richardson et al., 1991; Sabaty et al., 1994).

The redox state of the Q pool modulates not only photosynthesis but also the activity of the terminal oxidases of aerobic respiration (Zannoni and Moore, 1990). While electron transport through the cytochrome bc_1-C*c*O pathways is linearly related to the redox state of the Q pool (between 4% and 30% of Q reduction), electron flux through the quinol oxidase pathway is strongly limited until the Q pool is ~25 % reduced. Based on a Q pool formed by approximately 60 Q per RC, it has been estimated that the K_m of QH_2 at the Q_o site of the cytochrome bc_1 complex (Cramer and Knaff, 1990) is equivalent to 2.4 to 3 QH_2 per RC, while at the Q_o site of the QH_2-bb_3 pathway (quinol oxidase), the K_m of QH_2 is close to 15 QH_2 per RC (Zannoni and Moore, 1990). Thus, the quinol oxidase pathway has a lower affinity for QH_2 than the cytochrome bc_1-C*c*O segment of the chain.

In conclusion, the organization of the bioenergetic systems in facultative phototrophs satisfies two op-

posing needs, namely 1) the photosynthetic apparatus has to interact with the aerobic and/or anaerobic respiratory chains for a better use of the available energy, and 2) this latter interaction must prevent the over-reduction of the photocyclic chain at the level of quinone to avoid inhibition of photosynthesis. In this respect, it is worth noting the recent findings that the membrane associated thiol:disulfide oxidoreductase (DsbB) of *Rba. capsulatus* may allow oxidation of the membrane embedded quinols in the presence of the water soluble metalloid tellurite (TeO_3^{2-}) (Borsetti et al., 2007). This finding suggests that metalloids might also support cell growth under unfavorable reducing conditions often experienced by facultative phototrophs (see Chapter 33, Borsetti et al.).

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References

Ädelroth P and Brzezinski P (2004) Surface-mediated proton-transfer reactions in membrane-bound proteins. Biochim Biophys Acta 1655: 102–115

Ädelroth P and Hosler JP (2006) Surface proton donors for the D-pathway of cytochrome *c* oxidase in the absence of subunit III. Biochemistry 45: 8308–8318

Ädelroth P, Gennis RB and Brzezinski P (1998) Role of the pathway through K(I-362) in proton transfer in cytochrome *c* oxidase from *R. sphaeroides*. Biochemistry 37: 2470–2476

Afkar E, Lisak J, Saltikov C, Basu P, Oremland RS and Stolz JF (2003) The respiratory arsenate reductase from *Bacillus selenitireducens* strain MLS10. FEMS Microbiol Lett 226: 107–112

Anderson G, Williams J and Hille R (1992) The purification and characterization of arsenite oxidase from *Alcaligenes faecalis*, a molybdenum-containing hydroxylase. J Biol Chem 267: 23674–23682

Arata H, Shimizu M and Takamiya K (1992) Purification and properties of trimethylamine-N-oxide reductase from the aerobic photosynthetic bacterium *Roseobacter denitrificans*. J Biochem 112: 470–475

Babcock GT (1999) How oxygen is activated and reduced in respiration. Proc Natl Acad Sci USA 96: 12971–12973

Baymann F, Brugna M, Muhlenhoff U and Nitschke W (2001) Daddy, where did (PS)I come from? Biochim Biophys Acta 1507: 291–310

Baymann F, Lebrun E, Brugna M, Schoepp-Cothenet B, Giudici-Orticoni M-T and Nitschke W (2003) The redox protein construction kit: Pre-last universal common ancestor evolution of energy-conserving enzymes. Phil Trans Roy Soc Lond B 358: 267–274

Bender KS, Shang C, Chakraborty R, Belchik SM, Coates JD and Achenbach LA (2005) Identification, characterization, and classification of genes encoding perchlorate reductase. J Bacteriol 187: 5090–5096

Bertero MG, Rothery RA, Palak M, Hou C, Lim D, Blasco F, Weiner JH and Strynadka NCJ (2003) Insights into the respiratory electron transfer pathway from the structure of nitrate reductase A. Nature Struct Biol 10: 681–687

Blankenship RE and Hartman H (1998) The origin and evolution of oxygenic photosynthesis. Trends Biochem Sci 23: 94–97

Blasco G, Guigliarelli B, Magalon A, Asso M, Giordano G and Rothery RA (2001) The coordination and function of the redox centers of the membrane bound nitrate reductases. Cell Molec Life Sci 58: 179–193

Bonora P, Principi I, Hochkoeppler A, Borghese R and Zannoni D (1998) The respiratory chain of the halophilic anoxygenic purple bacterium *Rhodospirillum sodomense*. Arch Microbiol 170: 435–441

Borisov VB, Liebl U, Rappaport F, Martin JL, Zhang J, Gennis RB, Konstantinov AA and Vos MH (2002) Interactions between heme *d* and heme b_{595} in quinol oxidase *bd* from *Escherichia coli*: A photoselection study using femtosecond spectroscopy. Biochemistry 41: 1654–1662

Borsetti F, Francia F, Turner RJ and Zannoni D (2007) The thiol: disulfide oxidoreductase DsbB mediates the oxidizing effects of the toxic metalloid tellurite on the plasma membrane redox system of the facultative phototroph *Rhodobacter capsulatus*. J Bacteriol 189: 851–859

Branden G, Gennis RB and Brzezinski P (2006a) Transmembrane proton translocation by cytochrome *c* oxidase. Biochim Biophys Acta 1757: 1052–1063

Branden G, Pawate AS, Gennis RB and Brzezinski P (2006b) Controlled uncoupling and recoupling of proton pumping in cytochrome *c* oxidase. Proc Natl Acad Sci USA 103: 317–322

Brasier MD, Green OR, Jephcoat AP, Kleppe AK, Van Kranendonk MJ, Lindsay JF, Steele A and Grassineau NV (2002) Questioning the evidence for Earth's oldest fossils. Nature 616: 76–81

Bratton MR, Pressler MA and Hosler JP (1999) Suicide inactivation of cytochrome *c* oxidase: Catalytic turnover in the absence of subunit III alters the active site. Biochemistry 38: 16236–16245

Bratton MR, Hiser L, Antholine WE, Hoganson C and Hosler JP (2000) Identification of the structural subunits required for formation of the metal centers in subunit I of cytochrome *c* oxidase of *Rhodobacter sphaeroides*. Biochemistry 39: 12989–12995

Brochier C and Philippe H (2002) Phylogeny: A non hyperthermophilic ancestor for bacteria. Nature 417:244

Candela M, Zaccherini E and Zannoni D (2001) Respiratory electron transport and light induced energy transduction in membranes from the photosynthetic bacterium *Roseobacter denitrificans*. Arch Microbiol 175: 169–177

Castresana J (2001) Comparative genomics and bioenergetics. Biochim Biophys Acta 1506: 147–162

Cavalier-Smith T (2001) Obcells as proto-organisms: Membrane heredity, lithophosphorylation, and the origins of the genetic code, the first cell, and photosynthesis. J Mol Evol 53: 555–595

Ciurli S and Musiani F (2005) High potential iron-sulfur proteins and their role as soluble electron carriers in bacterial photosynthesis: Tale of a discovery. Photosynth Res 85: 115–131

Cotton NJP, Clark AJ and Jackson JB (1983) Interaction between the respiratory and photosynthetic electron transport chains of intact cells of *Rhodopseudomonas capsulata* mediated by membrane potential. Eur J Biochem 130: 581–587

Cox RL, Patterson C and Donohue TJ (2001) Roles for the *Rhodobacter sphaeroides* CcmA and CcmG proteins. J Bacteriol 183: 4643–4647

Cramer WA and Knaff DB (1990) Energy Transduction in Biological Membranes. A Textbook of Bioenergetics. Springer-Verlag, New York

Daldal F, Mandaci S, Winterstein C, Myllykallio H, Duyck K and Zannoni D (2001) Mobile cytochrome c_2 and membrane-anchored cytochrome c_y are both efficient electron donors to the cbb_3- and aa_3-type cytochrome *c* oxidases during respiratory growth of *Rhodobacter sphaeroides*. J Bacteriol 183: 2013–2024

Danielsson Thorell H, Beyer NH, Heegaard NH, Ohman M and Nilsson T (2004) Comparison of native and recombinant chlorite dismutase from *Ideonella dechloratans*. Eur J Biochem 271: 3539–3346

D'Mello R, Hill S and Poole RK (1994) Determination of the oxygen affinities of terminal oxidases in *Azotobacter vinelandii* using the deoxygenation of oxyleghaemoglobin and oxymyoglobin: Cytochrome *bd* is a low affinity oxidase. Microbiology 140: 1395–1402

D'Mello R, Hill S and Poole RK (1996) The cytochrome *bd* quinol oxidase in *Escherichia coli* has an extremely high oxygen affinity and two oxygen-binding haems: Implications for regulation of activity in vivo by oxygen inhibition. Microbiology 142: 755–763

Drosou V, Reincke B, Schneider M and Ludwig B (2002) 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: 10629–10634

Dueweke TJ and Gennis RB (1991) Proteolysis of the cytochrome *d* complex with trypsin and chymotrypsin localizes a quinol oxidase domain. Biochemistry 30: 3401–3406

Dupuis A, Prieur I and Lunardi J (2001) Toward a characterization of the connecting module of complex I. J Bioenerg Biomembr 33: 159–168

Ellis PT, Conrads T, Hille R and Kuhn P (2001) Crystal structure of the 100 kDa arsenite oxidase from *Alcaligenes faecalis* in two crystal forms at 1.6 Å and 2.03 Å. Structure 9: 125–132

Ferguson SJ and Richardson DJ (2004) The enzymes and bioenergetics of bacterial nitrate, nitrite, nitric oxide and nitrous oxide respiration. In: Zannoni D (ed) Respiration in Archaea and Bacteria. Diversity of Prokaryotic Respiratory Systems (Advances in Photosynthesis and Respiration, Vol 16), pp 169–206 Springer, Dordrecht

Fetter JR, Qian J, Shapleigh J, Thomas JW, Garcia-Horsman A, Schmidt E, Hosler J, Babcock GT, Gennis RB and Ferguson-Miller S (1995) Possible proton relay pathways in cytochrome *c* oxidase. Proc Natl Acad Sci USA 92: 1604–1608

Finel M (1996) Genetic inactivation of the H^+-translocating NADH:ubiquinone oxidoreductase of *Paracoccus denitrificans* is facilitated by insertion of the *ndh* gene from *Escherichia coli*. FEBS Lett 393: 81–85

Friedrich T and Scheide D (2000) The respiratory complex I of bacteria, archaea and eukarya and its module common with membrane-bound multisubunit hydrogenases. FEBS Lett 479: 1–5

García-Horsman JA, Barquera B and Escamilla JE (1991) Two different aa_3-type cytochromes can be purified from the bacterium *Bacillus cereus*. Eur J Biochem 199: 761–768

García-Horsman JA, Barquera B, Rumbley J, Ma J and Gennis R (1994) The superfamily of heme-copper respiratory oxidases. J Bacteriol. 176: 5587–5600

Gilderson G, Salomonsson L, Aagaard A, Gray J, Brzezinski P and Hosler J (2003) Subunit III of cytochrome *c* oxidase of *Rhodobacter sphaeroides* is required to maintain rapid proton uptake through the D pathway at physiologic pH. Biochemistry 42: 7400–7409

Gon S, Giudici-Orticoni MT, Mejean V and Iobbi-Nivol C (2001) Electron transfer and binding of the *c*-type cytochrome TorC to the trimethylamine-N-oxide reductase of *Escherichia coli*. J Bacteriol 182: 5779–5786

Hanlon SP, Holt RA, Moore GR and McEwan AG (1994) Isolation and characterization of a strain of *Rhodobacter sulfidophilus*: A bacterium which grows autotrophically with dimethylsulfide as electron donor. Microbiology 140: 1953–1958

Hemp J, Christian C, Barquera B, Gennis RB and Martinez TJ (2005) Helix switching of a key active-site residue in the cytochrome cbb_3 oxidases. Biochemistry 44: 10766–10775

Hemp J, Robinson DE, Ganesan KB, Martinez TJ, Kelleher NL and Gennis RB (2006) Evolutionary migration of a post-translationally modified active-site residue in the proton-pumping heme-copper oxygen reductases. Biochemistry 45: 15405–15410

Herter SM, Kortlüle CM and Drews G (1998) Complex I of *Rhodobacter capsulatus* and its role in reverted electron transport. Arch Microbiol 169: 98–105

Hill BC (1994) Modeling the sequence of electron transfer reactions in the single turnover of reduced, mammalian cytochrome *c* oxidase with oxygen. J Biol Chem 269: 2419–2425

Hiser L, Di Valentin M, Hamer AG and Hosler JP (2000) Cox11p is required for stable formation of the Cu_B and magnesium centers of cytochrome *c* oxidase. J Biol Chem 275: 619–623

Hochkoeppler A, Ciurli S, Venturoli G and Zannoni D (1995a) The high potential iron-sulphur protein (HiPIP) from *Rhodoferax fermentans* is competent in photosynthetic electron transfer. FEBS Lett 357:70–74

Hochkoeppler A, Jenney FE, Jr., Lang SE, Zannoni D and Daldal F (1995b) Membrane-associated cytochrome c_y of *Rhodobacter capsulatus* is an electron carrier from the cytochrome bc_1 complex to the cytochrome *c* oxidase during respiration. J Bacteriol 177: 608–613

Hochkoeppler A, Moschettini G and Zannoni D (1995c) The electron transport system of the facultative phototrophic bacterium *Rhodoferax fermentans*. I. A functional, thermodynamic and spectroscopic study of the membrane bound respiratory chain of dark- and light-grown cells. Biochim Biophys Acta 1229:73–80

Hochkoeppler A, Principi I, Bonora P, Ciurli S and Zannoni D (1999) On the role of soluble redox carriers alternative to cytochrome c_2 as donors to tetraheme-type reaction centers and cytochrome oxidases. In: Peschek GA, Loffelhardt W and Schmetterer G (eds) The Phototrophic Prokaryotes, pp 293–302. Kluwer Academic / Plenum Publishers, New York

Holland HD (1994) Early proterozoic atmospheric change. In: Bengtson S (ed) Early Life on Earth, pp 237–244. Columbia University Press, New York

Hosler JP (2004) The influence of subunit III of cytochrome *c* oxidase on the D pathway, the proton exit pathway and mechanism-based inactivation in subunit I. Biochim Biophys Acta 1655: 332–339

Hosler JP, Ferguson-Miller S, Calhoun MW, Thomas JW, Hill J, Lemieux L, Ma J, Georgiou C, Fetter J, Shapleigh J, Teklenburg MMJ, Babcock GT and Gennis RB (1993) Insight into the active-site structure and function of cytochrome oxidase by analysis of site-directed mutants of bacterial cytochrome aa_3 and cytochrome *bo*. J Bioenerg Biomembr 25: 121–136

Hosler JP, Ferguson-Miller S and Mills DA (2006) Energy transduction: Proton transfer through the respiratory complexes. Annu Rev Biochem 75: 165–187

Imhoff JF, Petri R and Suling J (1998) Reclassification of species of the spiral-shaped phototrophic purple non-sufur bacteria of the alpha-Proteobacteria: Description of the new genera *Paeospirillum* gen. nov., *Rhodovibrio* gen. nov., *Rhodothalassium* gen. nov. and *Roseospira* gen. nov. as well as transfer of *Rhodospirillum fulvum* to *Phaeospirillum fulvum* comb. nov., of *Rhodospirillum molischianum* to *Paeospirillum molischianum* com. nov., of *Rhodospirillum salinarum* to *Rhodovibrio salexigens*. Int J Syst Bacteriol 48: 793–798

Iobbi-Nivol C, Pommier J, Simala-Grant J, Mejean V and Giordano G (1996) High substrate specificity and induction characteristics of trimethylamine-N-oxide reductase of *Escherichia coli*. Biochim Biophys Acta 1294: 157–162

Jenney Jr. FE and Daldal F (1993) A novel membrane-associated *c*-type cytochrome Cyt c_y, can mediate the photosynthetic growth of *Rhodobacter capsuatus* and *Rhodobacter sphaeroides*. EMBO J 12: 1283–1292

Johnson KE and Rajagopalan KV (2001) An active site tyrosine influences the ability of the dimethylsulfoxide reductase family of molybdopterin enzymes to reduce S-oxides. J Biol Chem 276: 13178–13185

Jormakka M, Richardson D, Byrne B and Iwata S (2004) Architecture of NarGH reveals a structural classification of Mo-bisMGD enzymes. Structure 12: 95–104

Junemann S (1997) Cytochrome *bd* terminal oxidase. Biochim Biophys Acta 1321: 107–127

Jungst A, Wakabayashi S, Matsubara H and Zunft WG (1991) The *nirSTBM* region coding for cytochrome cd_1-dependent nitrite respiration in *Pseudomonas stutzeri* consists of a cluster of mono-, di-, and tetraheme proteins. FEBS Lett 279: 205–209

Kelly DP and Smith NA (1990) Organic sulphur compounds in the environment-biogeochemistry, microbiology, and ecological aspects. Adv Microb Ecol 11: 345–385

Kloer DP, Hagel C, Heider J and Schulz GE (2006) Crystal structure of ethylbenzene dehydrogenase from *Aromatoleum aromaticum*. Structure 14: 1377–1388

Kniemeyer O and Heider J (2001) Ethylbenzene dehydrogenase, a novel hydrocarbon-oxidizing molybdenum/iron-sulfur/heme enzyme. J Biol Chem 276: 21381–21386

Koch HG, Hwang O and Daldal F (1998) Isolation and characterization of *Rhodobacter capsulatus* mutants affected in cytochrome cbb_3 oxidase activity. J Bacteriol 180: 969–978

Krafft T and Macy JM (1998) Purification and characterization of the respiratory arsenate reductase of *Chrysiogenes arsenatis*. Eur J Biochem 255: 647–653

Krafft T, Bokranz M, Klimmer O, Schröder I, Fahrenholz F, Kojro E and Kröger A (1992) Cloning and nucleodide sequence of the *psrA* gene of *Wolinella succinogenes* polysulphide reductase. Eur J Biochem 206: 503–510

Lebrun E, Brugna M, Baymann F, Muller D, Lievremont D, Lett M-C and Nitschke W (2003) Arsenite oxidase, an ancient bioenergetic enzyme. Mol Biol Evol 20: 686–693

Lebrun E, Santini JM, Brugna M, Ducluzeau AL, Ouchane S, Schoepp-Cothenet B, Baymann F and Nitschke W (2006) The Rieske-protein: A case study on the pitfalls of multiple sequence alignments and phylogenetic reconstruction. Mol Biol Evol 23: 1180–1191

Lemon DD, Calhoun MW, Gennis RB and Woodruff WH (1993) The gateway to the active site of heme-copper oxidases. Biochemistry 32: 11953–11956

Madigan MT, Martinko JM and Parker J (1997) Brock. Biology of Microorganisms. 10th Edition. Printice Hall, Upper Saddle River

Mackenzie C, Choudhary M, Larimer FW, Predki PF, Stilwagen S, Armitage JP, Barber RD, Donohue TJ, Hosler JP, Newman JE, Shapleigh JP, Sockett RE, Zeilstra-Ryalls J and Kaplan S (2001) The home stretch, a first analysis of the nearly completed genome of *Rhodobacter sphaeroides* 2.4.1. Photosynth Res 70: 19–41

McDevitt CA, Hugenholtz P, Hanson GR and McEwan AG (2002a) Molecular analysis of dimethylsulfide dehydrogenase from *Rhodovulum sulfidophilum*; its place in the DMSO reductase family of microbial molybdenum-containing enzymes. Mol Microbiol 44: 1576–1587

McDevitt CA, Hanson GR, Noble CJ, Cheesman MR and McEwan AG (2002b) Characterization of the redox centers in dimethyl sulfide dehydrogenase from *Rhodovulum sulfidophilum*. Biochemistry 41: 15234–15244

McEwan AG, Cotton NPJ, Ferguson SJ and Jackson JB (1985) The role of auxillary oxidants in the maintenance of balanced redox poise for photosynthesis in bacteria. Biochim Biophys Acta 810: 140–147

McEwan AG, Ridge JP, McDevitt CA and Hugenholtz P (2002) The DMSO reductase family of microbial molybdenum enzymes; molecular properties and role in the dissimilatory reduction of toxic elements. Geomicrobiol J 19: 3–21

McEwan AG, Kappler U and McDevitt CA (2004) Microbial molybdenum-containing enzymes in respiration: structural and functional aspects. In: Zannoni D (ed) Respiration in Archaea and Bacteria. Diversity of Prokaryotic Electron Transport Carriers (Advances in Photosynthesis and Respiration, Vol 15), pp 175–202. Kluwer Academic Publishers, Dordrecht

Meyer TE and Cusanovich MA (1985) Soluble cytochrome composition of the purple phototrophic bacterium *Rhodopseudomas sphaeroides* ATCC 17023. Biochim Biophys Acta 807: 308–391

Meyer TE and Donohue TJ (1995) Cytochromes, iron-sulfur, and copper proteins mediating electron transfer from Cyt bc_1 complex to photosynthetic reaction center complexes. In: Blankenship RE, Madigan MT and Bauer CE (eds) Anoxygenic Photosynthetic Bacteria (Advances in Photosynthesis and Respiration, Vol 2), pp 725–745. Kluwer Academic Publishers, Dordrecht

Mills DA, Schmidt B, Hiser C, Westley E and Ferguson-Miller S (2002) Membrane potential-controlled inhibition of cytochrome *c* oxidase by zinc. J Biol Chem. 277: 14894–14901

Mills DA and Hosler JP (2005) Slow proton transfer through

the pathways for pumped protons in cytochrome *c* oxidase induces suicide inactivation of the enzyme. Biochemistry 44: 4656–4666

Mills DA, Tan Z, Ferguson-Miller S and Hosler J (2003) A role for subunit III in proton uptake into the D pathway and a possible proton exit pathway in *Rhodobacter sphaeroides* cytochrome *c* oxidase. Biochemistry 42: 7410–7417

Mills DA, Geren L, Hiser C, Schmidt B, Durham B, Millett F and Ferguson-Miller S (2005) An arginine to lysine mutation in the vicinity of the heme propionates affects the redox potentials of the hemes and associated electron and proton transfer in cytochrome *c* oxidase. Biochemistry 44: 10457–10465

Mogi T, Akimoto S, Endou S, Watanabe-Nakayama T, Mizuochi-Asai E and Miyoshi H (2006) Probing the ubiquinol-binding site in cytochrome *bd* by site-directed mutagenesis. Biochemistry 45: 7924–7930

Moore MD and Kaplan S (1992) Identification of intrinsic high-level resistance to rare-earth oxides and oxyanions in members of the class *Proteobacteria*: Characterization of tellurite, selenite, and rhodium sesquioxide reduction in *Rhodobacter sphaeroides*. J Bacteriol 174: 1505–1514

Morgan JE, Verkhovsky MI, Palmer G and Wikström M (2001) Role of the Pr intermediate in the reaction of cytochrome *c* oxidase with O_2. Biochemistry 40: 6882–6892

Moschettini G, Bonora P, Zaccherini E, Hochkoeppler A, Principi I and Zannoni D (1999) The primary quinone acceptor and the membrane-bound *c*-type cytochromes of the halophilic purple nonsulfur bacterium *Rhodospirillum salinarum*: a spectroscopic and thermodynamic study. Photosynth Research 62:43–53

Mouncey NJ, Gak E, Choudhary M, Oh J and Kaplan S (2000) Respiratory pathways of *Rhodobacter sphaeroides* $2.4.1^T$: Identification and characterization of genes encoding quinol oxidases. FEMS Microbiol Lett 192: 205–210

Mukhopadhyay R, Rosen BP, Phung LT and Silver S (2002) Microbial arsenic: From geocycles to genes and enzymes. FEMS Microbiol Rev 26: 311–325

Muller D, Lièvremont D, Dancheva Simeonova D, Hubert J-C and Lett M-C (2003) Arsenite oxidase *aox* genes from a metal-resistant !-proteobacterium. J Bacteriol 185: 135–141

Namslauer A and Brzezinski P (2004) Structural elements involved in electron-coupled proton transfer in cytochrome *c* oxidase. FEBS Lett. 567: 103–110

Nitschke W, Muhlenhoff U and Liebl U (1997) Evolution. In: Raghavendra A (ed) Photosynthesis: A Comprehensive Treatise, pp 285–304. Cambridge University Press, Cambridge

Oh JI (2006) Effect of mutations of five conserved histidine residues in the catalytic subunit of the cbb_3 cytochrome *c* oxidase on its function. J Microbiol 44: 284–292

Oh JI and Kaplan S (1999) The cbb_3 terminal oxidase of *Rhodobacter sphaeroides* 2.4.1: Structural and functional implications for the regulation of spectral complex formation. Biochemistry 38: 2688–2696

Oh JI and Kaplan S (2002) Oxygen adaptation. The role of the CcoQ subunit of the cbb_3 cytochrome *c* oxidase of *Rhodobacter sphaeroides* 2.4.1. J Biol Chem 277: 16220–16228

Oh JI, Ko IJ and Kaplan S (2004) Reconstitution of the *Rhodobacter sphaeroides* cbb_3-PrrBA signal transduction pathway in vitro. Biochemistry 43: 7915–7923

Ohmoto H (1996) Evidence in pre-2.2 Ga paleosols for the early evolution of atmospheric oxygen and terrestrial biota. Geology 24: 1135–1138

Ohmoto H (1997) When did the Earth's atmosphere become oxic? Geochemical News 93: 12–29

Olson JM (1999) Early evolution in chlorophyll-based photosynthesis. Chemtracts 12: 468–482

Oremland RS and Stolz JF (2003) The ecology of Arsenic. Science 300: 939–944

Oremland RS and Stolz JF (2005) Arsenic, microbes and contaminated aquifers. Trends in Microbiology 13: 45–49

Oremland RS, Blum JS, Culbertson CW, Visscher PT, Miller LG, Dowdle P and Strohmaier FE (1994) Isolation, growth, and metabolism of an obligately anaerobic, selenate-respiring bacterium, strain SES-3. Appl Environ Microbiol 60: 3011–3019

Oremland RS, Hoeft SE, Santini JM, Bano N, Hollibaugh RA and Hollibaugh RT (2002) Anaerobic oxidation of arsenite in Mono Lake water and by a facultative, arsenite-oxidizing chemoautotroph, strain MLHE-1. Appl Environ Microbiol 68: 4795–4802

Pappas CT, Sram J, Moskvin OV, Ivanov PS, Mackenzie RC, Choudhary M, Land ML, Larimer FW, Kaplan S and Gomelsky M (2004) Construction and validation of the *Rhodobacter sphaeroides* 2.4.1 DNA microarray: Transcriptome flexibility at diverse growth modes. J Bacteriol 186: 4748–4758

Pawate AS, Morgan J, Namslauer A, Mills D, Brzezinski P, Ferguson-Miller S and Gennis RB (2002) A mutation in subunit I of cytochrome oxidase from *Rhodobacter sphaeroides* results in an increase in steady-state activity but completely eliminates proton pumping. Biochemistry 41: 13417–13423

Pils D and Schmetterer G (2001) Characterization of three bioenergetically active respiratory terminal oxidases in the cyanobacterium *Synechocystis sp*. Strain PCC 6803. FEMS Microbiol Lett 203: 217–222

Pitcher RS and Watmough NJ (2004) The bacterial cytochrome cbb_3 oxidases. Biochim Biophys Acta 1655: 388–399

Poole RK, Lloyd D and Chance B (1979) The reaction of cytochrome oxidase with oxygen in the fission yeast *Schizosaccharomyces pombe* 972H-. Studies at subzero temperatures and measurement of apparent oxygen affinity. Biochem J 184: 555–563

Preisig O, Zufferey R, Thoeny-Meyer L, Appleby CA and Hennecke H (1996) A high-affinity cbb_3-type cytochrome oxidase terminates the symbiosis-specific respiratory chain of *Bradyrhizobium japonicum*. J Bacteriol 178: 1532–1538

Proshlyakov DA (2004) UV optical absorption by protein radicals in cytochrome *c* oxidase. Biochim Biophys Acta 1655: 282–289

Proshlyakov DA, Pressler MA and Babcock GT (1998) Dioxygen activation and bond cleavage by mixed-valence cytochrome *c* oxidase. Proc Natl Acad Sci USA 95: 8020–8025

Proshlyakov DA, Pressler MA, DeMaso C, Leykam JF, DeWitt DL and Babcock GT (2000) Oxygen activation and reduction in respiration: Involvement of redox-active tyrosine 244. Science 290: 1588–1591

Qin L, Hiser C, Mulichak A, Garavito RM and Ferguson-Miller S (2006) Identification of conserved lipid/detergent-binding sites in a high-resolution structure of the membrane protein cytochrome *c* oxidase. Proc Natl Acad Sci USA 103: 16117–16122

Rauhamaki V, Baumann M, Soliymani R, Puustinen A and Wikström M (2006) Identification of a histidine-tyrosine cross-link in the active site of the cbb_3-type cytochrome *c* oxidase from *Rhodobacter sphaeroides*. Proc Natl Acad Sci USA 103: 16135–16140

Reysenbach AL and Shock E (2002) Merging genomes with

geochemistry in hydrothermal ecosystems. Science 296: 1077–1082
Richardson DJ (2000) Bacterial respiration: a flexible process for a changing environment. Microbiology 146: 551–571
Richardson DJ, Bell LC, McEwan AG, Jackson JB and Ferguson SJ (1991) Cytochrome c_2 is essential for electron transfer to nitrous oxide reductase from physiological substrates in *Rhodobacter capsulatus* and acts as electron donor to the reductase *in vivo*. Correlation with photoinhibition studies. Eur J Biochem 199: 677–683
Richaud P, Marrs BL and Verméglio A (1986) Two modes of interaction between photosynthetic and respiratory electron chains in whole cells of *Rhodopseudomonas capsulata*. Biochim Biophys Acta 850: 256–263
Richter OM and Ludwig B (2003) Cytochrome *c* oxidase–structure, function, and physiology of a redox-driven molecular machine. Rev Physiol Biochem Pharmacol 147: 47–74
Riistama S, Puustinen A, Verkhovsky MI, Morgan JE and Wikström M (2000) Binding of O_2 and its reduction are both retarded by replacement of valine 279 by isoleucine in cytochrome *c* oxidase from *Paracoccus denitrificans*. Biochemistry 39: 6365–6372
Rott MA, Witthuhn VC, Schike BA, Soranno M, Ali A and Donohue TJ (1993) Genetic evidence for the role of isocytochrome c_2 in photosynthetic growth of *Rhodobacter sphaeroides* spd mutants. J Bacteriol 175: 358–366
Rugolo M and Zannoni D (1983) Oxygen induced inhibition of light dependent uptake of tetraphenylphosphonium ions as a probe of a direct interaction between photosynthetic and respiratory components in cells of *Rhodopseudomonas capsulata*. Biochem Biophys Res Commun 113:155–162
Ruitenberg M, Kannt A, Bamberg E, Ludwig B, Michel H and Fendler K (2000) Single-electron reduction of the oxidized state is coupled to proton uptake via the K pathway in *Paracoccus denitrificans* cytochrome *c* oxidase. Proc Natl Acad Sci USA 97: 4632–4636
Sabaty M, Gans P and Verméglio A (1993) Inhibition of nitrate reduction by light and oxygen in *Rhodobacter sphaeroides* forma sp. *denitrificans*. Arch Microbiol 159: 153–159
Sabaty M, Jappé J, Olive J and Verméglio A (1994) Organization of electron transfer components in *Rhodobacter sphaeroides* forma sp. *denitrificans*. Biochim Biophys Acta 1187: 313–323
Sabaty M, Avazéri C, Pignol D and Verméglio A (2001) Characterization of the reduction of selenate and tellurite by nitrate reductases. Appl Environ Microbiol 67: 5122–5126
Saitou N and Nei M (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol Biol Evol 4: 406–425
Santini JM and vanden Hoven RN (2004) Molybdenum-containing arsenite oxidase of the chemolithoautotrophic arsenite oxidizer NT-26. J Bacteriol 186: 1614–1619
Santini JM, Sly LI, Schnagl RD and Macy JM (2000) A new chemolithoautotropic arsenite-oxidizing bacterium isolated from a gold mine: Phylogenetic, physiological, and preliminary biochemical studies. App Env Microbiol 66: 92–97
Santini JM, Kappler U, Ward SA, Honeychurch MJ, van den Hoven RN and Bernhard PV (2007) The NT-26 cytochrome *c*552 and its role in arsenite oxidation. Biochim Biophys Acta 1767: 189–196
Sazanov LA and Hinchliffe P (2006) Structure of the hydrophilic domain of respiratory complex I from *Thermus thermophilus*. Science 311: 1430–1436
Shapleigh J, Hosler JP, Tecklenburg MJ, Ferguson-Miller S, Babcock GT and Gennis RB (1992) Identification of the heme axial ligands for the cytochrome *a* component of cytochrome *c* oxidase. Proc Natl Acad Sci USA 89: 4786–4790
Sharma V, Puustinen A, Wikström M and Laakkonen L (2006) Sequence analysis of the cbb_3 oxidases and an atomic model for the *Rhodobacter sphaeroides* enzyme. Biochemistry 45: 5754–5765
Sharpe M, Qin L and Ferguson-Miller S (2005) Proton entry, exit and pathways in cytochrome oxidase: Insight from 'conserved' water. In: Wikström M (ed) Biophysical and Structural Aspects of Bioenergetics, pp 26–54. RSC Publishing, Cambridge
Shaw AL, Hanson GR and McEwan AG (1996) Cloning and sequence analysis of the dimethylsulfoxide reductase structural gene from *Rhodobacter capsulatus*. Biochim Biophys Acta 1276: 176–180
Shaw AL, Hochkoeppler A, Bonora P, Zannoni D, Hanson GR and McEwan AG (1999a) Characterization of DorC from *Rhodobacter capsulatus*, a *c*-type cytochrome involved in electron transfer to dimethylsulfoxide reductase. J Biol Chem 274: 9911–9914
Shaw AL, Leimkuehler S, Klipp W, Hanson GR and McEwan AG (1999b) Mutational analysis of the dimethylsulfoxide respiratory (*dor*) operon of *Rhodobacter capsulatus*. Microbiol 145: 1409–1420
Schopf JW, Kudryavtsev AB, Agresti DG, Wdowiak TJ and Czaja AD (2002) Laser-Raman imagery of Earth's earliest fossils. Nature 416: 73–76
Schröder I, Rech S, Krafft T and Macy JM (1997) Purification and characterization of the selenate reductase from *Thauera selenatis*. J Biol Chem 272: 23765–23768
Smith D, Gray J, Mitchell L, Antholine WE and Hosler JP (2005) Assembly of cytochrome *c* oxidase in the absence of assembly protein Surf1p leads to loss of the active site heme. J Biol Chem 280: 17652–17656
Stiburek L, Vesela K, Hansikova H, Pecina P, Tesarova M, Cerna L, Houstek J and Zeman J (2005) Tissue-specific cytochrome *c* oxidase assembly defects due to mutations in Sco2 and Surf1. Biochem J 392: 625–632
Stolz JF and Oremland RS (1999) Bacterial respiration of arsenic and selenium. FEMS Microbiol Rev 23: 615–627
Stolz JF, Basu P, Santini JM and Oremland RS (2006) Arsenic and selenium in microbial metabolism. Annu Rev Microbiol 60: 107–130
Svensson-Ek M, Abramson J, Larsson G, Tornroth S, Brzezinski P and Iwata S (2002) The X-ray crystal structures of wild-type and EQ(I-286) mutant cytochrome *c* oxidases from *Rhodobacter sphaeroides*. J Mol Biol 321: 329–339
Swem LR, Gong X, Yu CA and Bauer CE (2006) Identification of a ubiquinone-binding site that affects autophosphorylation of the sensor kinase RegB. J Biol Chem 281: 6768–6775
Switzer Blum J, Burns Bindi A, Buzzeli J, Stolz JF and Oremland RS (1998) *Bacillus arsenicoselenatis*, sp. nov., and *Bacillus selenitireducens*, sp. nov.: Two haloalkaliphiles from Mono Lake, California that respire oxyanions of selenium and arsenic. Arch Microbiol 171: 19–30
Takamiya K, Iba K and Okamura K (1987) Reaction center complex from an aerobic photosynthetic bacterium *Erythrobacter* species OCh114. Biochim Biophys Acta 890: 127—133
Taylor BF and Kiene RP (1989) Microbial-metabolism of dimeth-

ylsulfide. ACS Symposium Series 393: 202–221
Thöny-Mayer L (1997) Biogenesis of respiratory cytochromes in bacteria. Microbiol Mol Biol Rev 61: 337–376
Toledo-Cuevas M, Barquera B, Gennis RB, Wikström M and García-Horsman JA (1998) The cbb_3-type cytochrome *c* oxidase from *Rhodobacter sphaeroides*, a proton-pumping heme-copper oxidase. Biochim Biophys Acta 1365: 421–434
Tomson FL, Morgan JE, Gu G, Barquera B, Vygodina TV and Gennis RB (2003) Substitutions for glutamate 101 in subunit II of cytochrome *c* oxidase from *Rhodobacter sphaeroides* result in blocking the proton-conducting K-channel. Biochemistry 42: 1711–1717
Towe KM (1990) Aerobic respiration in the Archaean? Nature 348: 54–56
Towe KM (1994) Earth's early atmosphere: Constraints and opportunities for early evolution. In: Bengston S (ed) Early Life on Earth, pp. 36–47. Columbia University Press, New York
Towe KM (1996) Environmental oxygen conditions during the origina and early evolution of life. Adv Space Res 18: 7–15
Trieber CA, Rothery RA and Weiner JH (1996) Engineering a novel iron-sulfur cluster into the catalytic subunit of *Escherichia coli* dimethyl-sulfoxide reductase. J Biol Chem 271: 4620–4626
Unemoto T and Hayashi M (1993) Na^+-translocating NADH-quinone reductase of marine and halophilic bacteria. J Bioenerg Biomembr 25: 385–391
vanden Hoven RN and Santini JM (2004) Arsenite oxidation by the heterotroph *Hydrogenophaga* sp. str. NT-14: the arsenite oxidase and its physiological electron acceptor. Biochim Biophys Acta 1656: 148–155
Varanasi L, Mills D, Murphree A, Gray J, Purser C, Baker R and Hosler J (2006) Altering conserved lipid binding sites in cytochrome *c* oxidase of *Rhodobacter sphaeroides* perturbs the interaction between subunits I and III and promotes suicide inactivation of the enzyme. Biochemistry 45: 14896–14907
Verméglio A (1977) Secondary electron transfer in reaction centers of *Rhodopseudomonas sphaeroides*: out-of-phase periodicity of two for the formation of ubisemiquinone and fully reduced ubiquinone. Biochim Biophys Acta 459: 516–524
Visscher PT, Taylor BF and Kiene RP (1995) Microbial consumption of dimethyl sulfide and methanediol in coastal marine-sediments. FEMS Microbiol Ecol 18: 145–153
Visscher PT and Taylor BF (1993) Organic thiols as organolithotrophic substrates for growth of phototrophic bacteria. Appl Environ Microbiol 59: 93–96
Wang K, Zhen Y, Sadoski R, Grinnell S, Geren L, Ferguson-Miller S, Durham B and Millett F (1999) Definition of the interaction domain for cytochrome *c* on cytochrome *c* oxidase. I. Rapid kinetic analysis of electron transfer from cytochrome *c* to *Rhodobacter sphaeroides* cytochrome oxidase surface mutants. J Biol Chem 274: 38042–38050
Watts CA, Ridley H, Condie KL, Leaver JT, Richardson DJ and Butler CS (2003) Selenate reduction by *Enterobacter cloacae* SDL1a-1 is catalysed by a molybdenum-dependent membrane-bound enzyme that is distinct from the membrane-bound nitrate reductase. FEMS Microbiol Lett 228: 273–279
Watts CA, Ridley H, Dridge EJ, Leaver JT, Reilly AJ, Richardson DJ and Butler CS (2005) Microbial reduction of selenate and nitrate: common themes and variations. Biochem Soc Trans 33: 173–175
Wikström M (2004) Cytochrome *c* oxidase: 25 years of the elusive proton pump. Biochim Biophys Acta 1655: 241–247
Wood PM (1981) The redox potential of dimethylsulfoxide reduction to dimethylsulfide-evaluation and biochemical implications. FEBS Lett 124: 11–14
Wraight CA, Cogdell RJ and Chance B (1978) Ion transport and electrochemical gradients in photosynthetic bacteria. In: Clayton RK and Sistrom RW (eds) The Photosynthetic Bacteria, pp 471–502. Plenum Press, New York
Xiong J and Bauer CE (2002) Complex evolution of photosynthesis. Annu Rev Plant Biol 53: 503—521
Xu X and Yagi T (2001) Identification of the NADH binding subunit of energy transducing NADH-quinone oxidoreductase (NDH-1) of *Thermus thermophilus* HB-8. Biochem Biophys Res Commun 174: 667–672
Xu X, Matsuno-Yagi A and Yagi T (1991a) Characterization of the 25-kilodalton subunit of the energy transducing NADH-ubiquinone oxidoreductase of *Paracoccus denitrificans*: Sequence similarity to the 24-kilodalton subunit of the flavoprotein fraction of mammalian complex I. Biochemistry 30: 8678–8684
Xu X, Matsuno-Yagi A and Yagi T (1991b) The NADH-binding subunit of the energy transducing NADH-ubiquinone oxidoreductase of *Paracoccus denitrificans*: Gene cloning and deduced primary structure. Biochemistry 30: 6422–6428
Xu X, Matsuno-Yagi A and Yagi T (1992a) Gene cluster of the energy-transducing NADH-quinone oxidoreductase of *Paracoccus denitrificans*: Characterization of four structural gene products. Biochemistry 31: 6925–6932
Xu X, Matsuno-Yagi A and Yagi T (1992b) Structural features of the 66 kilodalton subunit of the energy transducing NADH-ubiquinone oxidoreductase (NDH-1) of *Paracoccus denitrificans*. Arch Biochem Biophys 296: 40–48
Xu X, Matsuno-Yagi A and Yagi T (1993) DNA sequencing of the seven remaining structural genes of the gene cluster encoding the energy-transducing NADH-quinone oxidoreductase of *Paracoccus denitrificans*. Arch Biochem Biophys 250: 302–311
Yagi T (1993) The bacterial energy-transducing NADH-quinone oxidoreductases. Biochim Biophys Acta 1141: 1–17
Yagi T and Matsuno-Yagi A (2003) The proton-translocating NADH-quinone oxidoreductase in the respiratory chain: The secret unlocked. Biochemistry 42: 2266–2274
Yagi T, Seo BB, Di Bernardo S, Nakamaru-Ogiso E, Kao MC and Matsuno-Yagi A (2001) NADH dehydrogenases: From basic science to biomedicine. J Bioenerg Biomembr 33: 233–242
Yoshikawa S, Shinzawa-Itoh K, Nakashima R, Yaono R, Yamashita E, Inoue N, Yao M, Fei MJ, Libeu CP, Mizushima T, Yamaguchi H, Tomizaki T and Tsukihara T (1998) Redox-coupled crystal structural changes in bovine heart cytochrome *c* oxidase. Science 280: 1723–1729
Yun CH, Beci R, Crofts AR, Kaplan S and Gennis RB (1990) Cloning and DNA sequencing of the *fbc* operon encoding the cytochrome bc_1 complex from *Rhodobacter sphaeroides*. Characterization of *fbc* deletion mutants and complementation by a site-specific mutational variant. Eur J Biochem 194: 399–411
Zannoni D (1995) Aerobic and anaerobic electron transport chains in anoxygenic phototrophic bacteria. In: Blankenship RE, Madigan MT and Bauer CE (eds) Anoxygenic Photosynthetic Bacteria (Advances in Photosynthesis and Respiration, Vol 2), pp 449–971. Kluwer Academic Publishers, Dordrecht
Zannoni D and Ingledew JW (1985) A thermodynamic analysis of the plasma membrane electron transport components in

phototrophically grown cells of *Chloroflexus aurantiacus*: An optical and electron paramagnetic resonance study. FEBS Letters 193: 93–98

Zannoni D and Moore AL (1990) Measurement of the redox state of the ubiquinone pool in *Rhodobacter capsulatus* membrane fragments. FEBS Letters 271:123–127

Zannoni D, Jasper P and Marrs BL (1978) Light induced oxygen uptake as a probe of electron transport between respiratory and photosynthetic components in membranes of *Rhodopseudomonas capsulata* Arch Biochem Biophys 191: 625–631

Zannoni D, Peterson S and Marrs BL (1986) Recovery of the alternative oxidase dependent electron flow by fusion of membrane vesicles from *Rhodobacter capsulatus* mutant strains. Arch Microbiol 144: 375–380

Zeller T, Moskvin OV, Li K, Klug G and Gomelsky M (2005) Transcriptome and physiological responses to hydrogen peroxide of the facultatively phototrophic bacterium *Rhodobacter sphaeroides*. J Bacteriol 187: 7232–7242

Zhang J, Barquera B and Gennis RB (2004) Gene fusions with beta-lactamase show that subunit I of the cytochrome *bd* quinol oxidase from *E. coli* has nine transmembrane helices with the O_2 reactive site near the periplasmic surface. FEBS Lett 561: 58–62

Zufferey R, Preisig O, Hennecke H and Thöny-Meyer L (1996) Assembly and function of the cytochrome cbb_3 oxidase subunits in *Bradyrhizobium japonicum*. J Biol Chem 271: 9114–9119