Biochimica et Biophysica Acta 1777 (2008) 1480–1490

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbabio

Review

The organisation of proton motive and non-proton motive redox loops in prokaryotic respiratory systems

Jörg Simon [a,b,*], Rob J.M. van Spanning [c], David J. Richardson [d,*]

[a] Institute of Molecular Biosciences, Goethe University, Max-von-Laue-Str. 9, 60438 Frankfurt am Main, Germany
[b] Department of Microbiology and Genetics, Technische Universität Darmstadt, Schnittspahnstr. 10, 64287 Darmstadt, Germany
[c] Department of Molecular Cell Physiology, VU University Amsterdam, NL-1081 HV Amsterdam, The Netherlands
[d] School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, UK

ARTICLE INFO

Article history:
Received 1 July 2008
Received in revised form 8 September 2008
Accepted 9 September 2008
Available online 30 September 2008

Keywords:
Aerobic and anaerobic respiration
Proton motive force
Redox (half) loop
Electron transport chain
Quinone reductase
Quinol dehydrogenase

ABSTRACT

Respiration is fundamental to the aerobic and anaerobic energy metabolism of many prokaryotic and most eukaryotic organisms. In principle, the free energy of a redox reaction catalysed by a membrane-bound electron transport chain is transduced via the generation of an electrochemical ion (usually proton) gradient across a coupling membrane that drives ATP synthesis. The proton motive force (*pmf*) can be built up by different mechanisms like proton pumping, quinone/quinol cycling or by a redox loop. The latter couples electron transport to a net proton transfer across the membrane without proton pumping. Instead, charge separation is achieved by quinone-reactive enzymes or enzyme complexes whose active sites for substrates and quinones are situated on different sides of the coupling membrane. The necessary transmembrane electron transport is usually accomplished by the presence of two haem groups that face opposite sides of the membrane. There are many different enzyme complexes that are part of redox loops and their catalysed redox reactions can be either electrogenic, electroneutral (non-proton motive) or even *pmf*-consuming. This article gives conceptual classification of different operational organisations of redox loops and uses this as a platform from which to explore the biodiversity of quinone/quinol-cycling redox systems.

1. Introduction

Respiration is a catabolic process that is fundamental to all kingdoms of life [1]. In human mitochondria, the ATP factories of our cells, electrons are extracted from organic carbon as it is catabolised through metabolic pathways such as glycolysis and the tricarboxylic acid cycle. The electrons are then passed via freely diffusible carriers, such as NADH, or protein-bound carriers, such as $FADH_2$, into a multi-protein electron transport pathway associated with the inner mitochondrial membrane (Fig. 1A). Here the electrons migrate through a range of electron transferring redox cofactors, such as flavins, iron sulfur clusters, haem and copper centres, that are bound to integral membrane or membrane-associated protein complexes, and ultimately reduce oxygen to water. Electrons that enter the electron transport pathway have a low electrochemical potential. For example, the midpoint redox potential (termed E_0' at pH 7.0) of the NAD^+/NADH redox couple is around −320 mV, thus making NADH a strong reductant. By contrast, the E_0' of the O_2/H_2O couple is around +820 mV, making it strongly oxidizing. Electrons thus flow 'downhill' in energy terms from NADH to oxygen and the free energy (ΔG) released during this electron transfer process is used to drive the translocation of protons across the inner mitochondrial membrane to generate a transmembrane electrochemical proton gradient or proton motive force (*pmf*) (Fig. 1A) [2–4]. The *pmf* has both a chemical (ΔpH, dimensionless) and an electrical (Δψ, dimension: mV) component. Eq. 1 shows the corresponding formula in its simplest form.

$$pmf\,(\mathrm{mV}) = \Delta\psi - 59\,\Delta\mathrm{pH} \qquad (1)$$

Here, Δψ is defined as the electrical potential difference between the positively and the negatively charged (P and N) side of the

Abbreviations: DMSO, dimethylsulfoxide; FAD/$FADH_2$, oxidised/reduced flavin adenine dinucleotide; Fe/S, iron sulfur cluster; H^+/e^-, ratio between the number of protons translocated across the coupling membrane and the amount of electrons transported in the accompanying redox reaction; MK/MKH_2, menaquinone/menaquinol; MKK, methyl-menaquinone; MP/MPH_2, methanophenazine/dihydro-methanophenazine; Mo-*bis*-MGD, molybdenum-*bis*-molybdopterin guanine dinucleotide; N, negatively charged side of the coupling membrane; NAD^+/NADH, oxidised/reduced nicotinamide adenine dinucleotide; Nap, periplasmic nitrate reductase system; Ni/Fe, catalytic Ni/Fe centre of hydrogenase; Nrf, cytochrome *c* nitrite reductase system; P, positively charged side of the coupling membrane; *pmf*, proton motive force; PQQ, pyrroloquinoline quinone; Q/QH_2, (ubi- or mena-) quinone/(ubi- or mena-) quinol; SQO, succinate:quinone oxidoreductase; TMAO, trimethylamine-*N*-oxide; TMS, transmembrane (α-helical protein) segment

* Corresponding authors. J. Simon is to be contacted at the Institute of Molecular Biosciences, Goethe University, Max-von-Laue-Str. 9, 60438 Frankfurt am Main, Germany. Tel.: +49 69 798 29516; fax: +49 69 798 29527. D.J. Richardson, School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, UK. Tel.: +44 1603 593250; fax: +44 1603 592250.

E-mail addresses: j.simon@bio.uni-frankfurt.de (J. Simon), d.richardson@uea.ac.uk (D.J. Richardson).

0005-2728/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbabio.2008.09.008

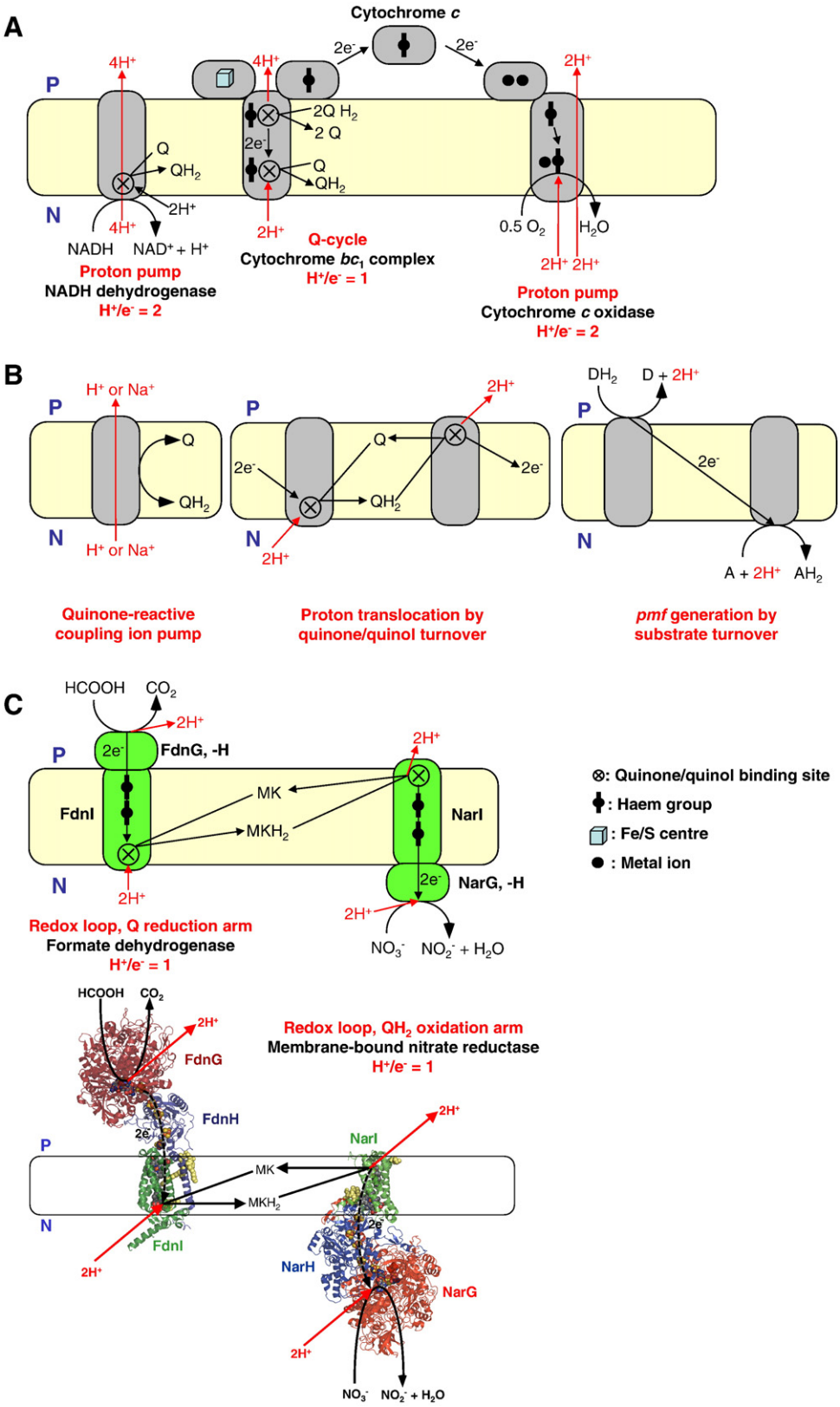

Fig. 1. Different mechanisms of *pmf* generation. (A) Schematic architecture of selected protein complexes involved in aerobic respiration, e.g. in mitochondria or in the bacterium *P. denitrificans*. (B) Dissection of principle mechanisms of *pmf* generation by intra- and interenzymic electron transport. Boxes represent electron transport enzymes or enzyme complexes in the membrane. Left, coupling ion pumping. Middle, proton translocation coupled to quinone redox chemistry. Right, *pmf* generation arising from divergent substrate site location without actual proton translocation. (C) The electron transport chain of anaerobic nitrate respiration with formate as electron donor in *E. coli* as a prototype of a two-enzyme full proton motive redox loop. The redox loop is a result of a combination of the proton translocation modes shown in the middle and on the right of (B). The structural models were prepared using PyMol and pdb files 1KQG and 1Q16. See Section 3 for details on enzyme architecture. P and N refer to the positively and negatively charged side of the membrane, respectively.

membrane and is usually positive. The ΔpH is defined as the pH difference between the P and the N side and is usually negative. Since the *pmf* has a value in the range of ~150–200 mV, a potential change (ΔE) of at least this magnitude during transfer from donor to acceptor is required if that step is to be coupled to *pmf* generation ($H^+/e^- = \Delta E/pmf$ at 100% thermodynamic efficiency). Overall, the transfer of 2 electrons from NADH to oxygen results in 10 positive charges being translocated across the membrane and there are three key proton motive steps that contribute to this: (I) the NADH dehydrogenase complex (NADH:ubiquinone oxidoreductase) which is a proton pump, (II) the cytochrome bc_1 complex (ubiquinone:cytochrome *c* oxidoreductase) that moves positive charge across the membrane via the so-called Q-cycle and (III) the cytochrome *c* oxidase (aa_3-type), which combines proton pumping with opposite electron and proton movements (Fig. 1A). This basic description of respiration is also fundamental to the bacterial and archaeal kingdoms of life, with one key difference. In *Bacteria* and *Archaea*, a diverse range of organic and inorganic substrates can be used to donate or accept electrons at various electrochemical potentials in order to drive aerobic or anaerobic respiratory electron transport systems [5].

In many mitochondrial and bacterial respiratory systems the link between the electron donating enzymes and the electron accepting enzymes is the quinone pool (Fig. 1). Quinones are small, freely diffusible, lipophilic, membrane-entrapped organic molecules that can carry 2 electrons and 2 protons when fully reduced, i.e. in the quinol state. Different kinds of quinones have different electrochemical potentials and many bacteria can synthesise more than one type of quinone. In general, where an organism can synthesise two quinone types, ubiquinone [E_0' (UQ/UQH_2) ~+40 mV] predominates under aerobic conditions and menaquinone [E_0' (MK/MKH_2) ~−80 mV] predominates under anaerobic conditions when the cellular state is more reduced. In certain *Archaea* the basic function of quinones is replaced by phenazine-based compounds. Inspection of Fig. 1 reveals that the active site of the respiratory enzymes for reductants and oxidants can be located at either the P or N side of the membrane across which the *pmf* is generated. This applies equally to the active sites for quinone and quinol turnover. The different locations of the active sites of respiratory enzymes in different cellular compartments introduce some rather interesting considerations for mechanisms by which electron transfer from donor to acceptor substrates can be coupled to *pmf* generation. These are addressed by the redox loop concept. The basic facet of a redox loop, as originally envisaged by the Nobel Laureate Peter Mitchell in his chemiosmotic hypothesis, was the separation of positive and negative charges across the energy-conserving membrane [6]. Such charge separations do not require the proteins involved to operate as proton pumps (Fig. 1B, left), but rather to operate as part of redox loops that provide electron-transferring molecular scaffolds across the membrane that facilitate the movement of electrons from the P to the N side of the membrane, hence contributing to the membrane potential component of the *pmf* through an electrogenic redox loop (Fig. 1B, middle and right). Such redox loops often consist of two quinone-reactive (multi-subunit) enzymes and a quinone species that serves as redox mediator between them (Fig. 1C; note that reactions catalysed by one such enzyme have been occasionally termed “proton motive redox half loop” or “electron-carrying arm of a redox loop”). In principle, the different localisation of the electron donor/acceptor and quinone/quinol centres allows for electrogenic, electroneutral [“non-proton motive redox (half) loop”] or even energy-dissipating reactions.

The great diversity in bacterial and archaeal respiration underlies the critical contribution of prokaryotes to the Earth's biogeochemistry. There are currently more than 600 complete or near-complete genome sequences of prokaryotic organisms, which provide a huge database to explore the biochemical diversity of mechanisms by which the ΔG released during respiratory electron transfer can be transduced in a *pmf*. Redox loops are commonly found in anaerobic respiratory systems where the ΔG value of the redox reaction is smaller than in aerobic respiration and where, consequently, shorter respiratory chains and less ion pumping enzymes are employed. The purpose of this article is to survey the possible mechanisms by which a *pmf* may conceptually be maintained by quinone-dependent respiratory systems and to assess how many such ‘conceptual systems’ have been identified or can be inferred from data currently available in the literature. The review focuses on the topological organisation of different quinone-reducing and quinol-oxidising systems in biological membranes of *Bacteria* and *Archaea* and discusses the consequences of this organisation for bioenergetics in terms of energy transduction or dissipation. Note that components of typical ‘mitochondrial-like’ aerobic respiratory chains will not be dealt with apart from succinate: quinone dehydrogenases. Likewise, established proton-pumping enzymes or systems using Na^+ as coupling ion are not within the scope of this article.

2. Configurations of quinone reducing and quinol oxidizing enzyme systems involved in redox loops

Conceptually, we propose that enzymes involved in redox loop mechanisms can be classified into at least nine operationally different types (designated ① to ⑨ in the schemes of Fig. 2 and exemplified in Table 1). These are differentiated on the basis of the location of the active sites for the electron donor or electron acceptor on the P or N sides of the membrane, the location of the quinone/quinol binding site on the P or N sides and the direction of electron transfer (P to N or N to P). If needed, transmembrane electron transport can be achieved by the presence of two haem *b* groups that are oriented towards different sides of the membrane (see configurations ①, ④, ⑤, ⑧ and ⑨ in Fig. 2).

2.1. Quinone reduction and donor:quinone dehydrogenase configurations

The conceivable configurations of donor:quinone dehydrogenases are shown in Fig. 2A (types ① to ④, see Table 1 for examples). The proton motive system of the formate dehydrogenase depicted in Fig. 1C falls into group ① ($H^+/e^- = 1$) in which the donor oxidation and quinone reduction sites are located at opposite sides of the membrane. As stated above, not all non-proton translocating quinone reducing systems are directly coupled to *pmf* generation. Type ② shows a non-proton motive enzyme in which both the donor oxidation site and quinone reduction site are located at the P side of the membrane ($H^+/e^- = 0$). An example of such an enzyme system would be the electron transport pathway from periplasmic hydroxylamine oxidoreductase via periplasmic cytochrome c_{554} to membrane-anchored cytochrome c_{M552} that couples hydroxylamine oxidation to ubiquinone reduction in nitrifying bacteria (Table 1). Note that the components of such an electron transport chain do not necessarily form a permanent membrane-bound complex and that, consequently, the quinone reductase could be the only membrane-integral protein. Another system where the donor dehydrogenase and quinone reductase site are located on the same side of the membrane occurs when they are both located on the N side (configuration ③). Despite being again non-proton motive ($H^+/e^- = 0$), it is rather commonly found, e.g. in the succinate dehydrogenase complex of mitochondria (complex II) and in functionally equivalent enzyme complexes of bacteria (Table 1). Finally, there is a configuration whereby the donor site is on the N side of the membrane and the quinone reduction site is on the P side (type ④). This system is dependent on the *pmf* and consumes it during catalysis ($H^+/e^- = -1$, “reverse redox loop”). Representatives for this mechanism are succinate:menaquinone dehydrogenases from Gram-positive bacteria and some sulfate reducers (e.g. *Desulfovibrio vulgaris*) whose membrane anchor subunits contain two haem *b* groups allowing for the functionally required transmembrane electron transport (Table 1).

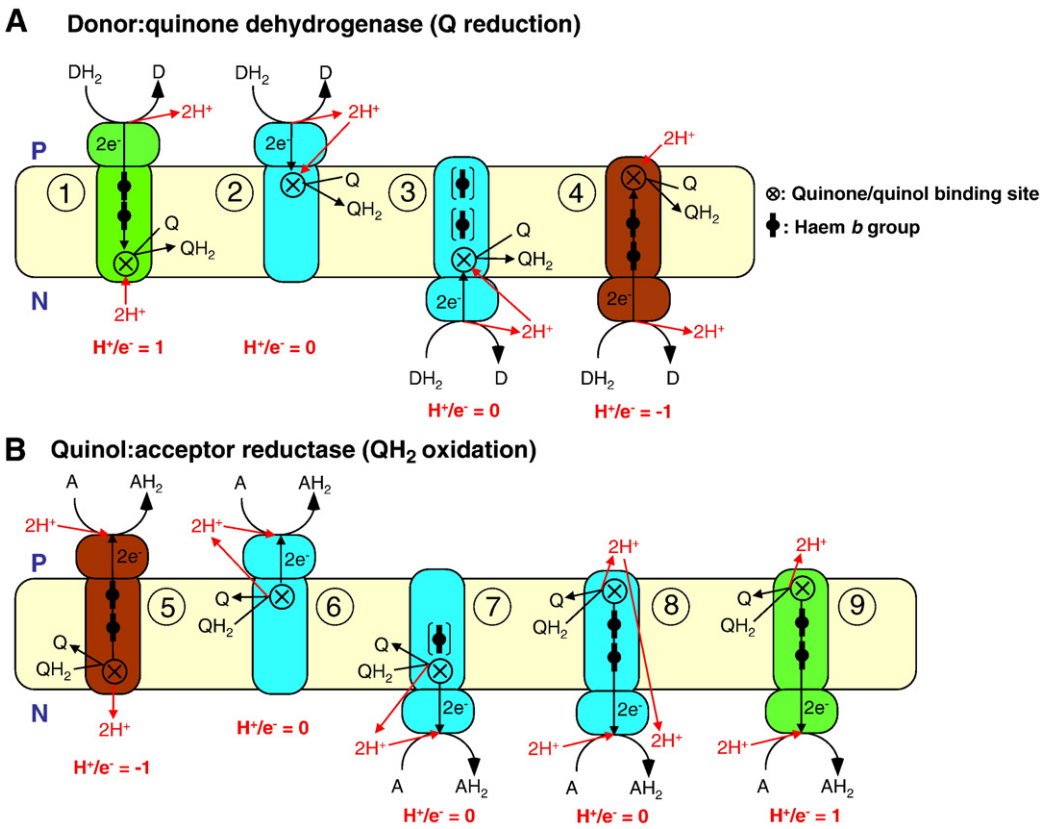

Fig. 2. Configurations of quinone-reducing (A) and quinol-oxidizing (B) electron transport enzymes. The enzymes are classified according to the binding site localisation for the electron donor (DH_2), electron acceptor (A), quinone (Q) and quinol (QH_2). Quinone-dependent substrate turnover results in either electrogenic (green, $H^+/e^-=1$), electroneutral (blue, $H^+/e^-=0$) or *pmf*-dissipating (red, $H^+/e^-=-1$) overall reactions. Prototypic enzymes or enzyme complexes exemplifying each configuration are presented in Table 1. In principle, quinone could be replaced by a phenazine (derivative) or a similar carrier. Haem *b* groups in brackets denote that these are not obligatorily present.

2.2. Quinol oxidation and quinol:acceptor reductase configurations

Conceptual arrangements for quinol oxidising systems are shown in Fig. 2B (types ⑤ to ⑨, see Table 1 for examples). Type ⑨ illustrates the example where quinol is oxidised at the P side and electrons are passed to the N side ($H^+/e^-=1$). This is the energy-conserving mechanism described for the membrane-bound nitrate reductase in Fig. 1C. In type ⑥, quinol is oxidised at the P side and the electrons pass to an acceptor site also located on the P side of the membrane. This type is found in many different electron transport systems and is exceptionally widespread in the prokaryotic world. Examples include the abundant NapC/NrfH, NapH and NrfD/PsrC families whose prototypic members function in periplasmic nitrate and nitrite reductase systems (Table 1) [7]. Type ⑦ illustrates an example of quinol oxidation and acceptor reduction both taking place on the N side of the membrane. An example of this is the structurally resolved menaquinol:fumarate reductase from *E. coli*. No *pmf* generation is associated with either type ⑥ or ⑦ since in both cases the negative and positive charges are not separated across the membrane. The configuration illustrated by type ⑧ resembles type ⑦, except that the quinol oxidation site is situated on the P side. Here, acceptor reduction by quinol, which in itself is electrogenic (resembling type ⑨), is accompanied by a compensatory proton transfer from the P to the N side making the overall reaction electroneutral ($H^+/e^-=0$). This is exemplified by the menaquinol:fumarate reductase complex from the ε-proteobacterium *Wolinella succinogenes* where the compensatory (i.e. uncoupling) proton flux is attained by the 'E-pathway' (see Mechanism D in section 3 for details). Finally, there is a configuration with the quinol site on the N side of the membrane and the electron acceptor site on the P side (type ⑤). This system would be *pmf*-consuming ($H^+/e^-=-1$) but there are currently no clear examples of this occurring.

3. The coupling of quinone reduction and quinol oxidation

Donor:quinone dehydrogenases and quinol:acceptor reductases have to be coupled together to create simple electron transport chains that produce a *pmf* by making use of proton motive redox (half) loop(s). With the quinone-reactive enzymes presented in Fig. 2, six different *pmf*-generating electron transport chains are conceivable and presented as mechanisms A–F in Fig. 3. Note that the *pmf*-consuming enzymes are not covered by these mechanisms since a respiratory chain that involves such an enzyme would need at least one proton-pump to make the overall reaction electrogenic. Accordingly, the type ④ succinate:menaquinone dehydrogenase of Gram-positive bacteria is part of the aerobic respiratory chain [8].

3.1. Mechanism A

This mechanism, depicted in Fig. 3A, is the full proton motive redox loop ($H^+/e^-=2$) that derives from coupling together two *pmf*-generating enzymes (types ① and ⑨) and cycling quinone/quinol between them. This mechanism is presented here in more detail with reference to two membrane-bound enzyme complexes from *E. coli*, the formate dehydrogenase (Fdh-N or FdnGHI complex) and nitrate reductase A (NarA or NarGHI complex) that together form the paradigmatic Fdh-Nar full redox loop (Fig. 1C) [9].

The *E. coli* NarA enzyme provides a classical example where a recently obtained crystal structure ultimately confirmed early

Table 1
Examples of the enzyme configurations presented in Fig. 2

Enzyme class	Quinone- or MP-reactive protein (subunit) and mode of membrane interaction[a]	Redox partner (cofactors), mode of interaction, representative organism(s) and enzyme (complex) designation[a]	References
Type ①	FdnI (FdhC/FdoI)		
	4 TMH (His: 1-1-0-2_{13})	FdnGH (FdhAB/FdoGH) (Mo, Fe/S); formation of membrane-bound formate dehydrogenase complex, *E. coli* (Fdh-N and Fdh-O); *W. succinogenes* (FdhABC)	[9, 51–53]
	HydC		
	4 TMS (His: 1-1-0-2_{13})	HydBA (Ni/Fe, Fe/S), formation of membrane-bound Ni/Fe-hydrogenase complex, *W. succinogenes* (HydABC)	[51,54]
	VhoC (VhtC)		
	5 TMS (His: 1-1-0-0-2_{13})	VhoAG (VhtAG) (Ni/Fe, Fe/S), formation of a membrane-bound and MP-reactive F_{420}-nonreducing hydrogenase complex. The presence of two haem *b* groups has not been shown experimentally. *Methanosarcina* spp.	[15]
Type ②	Cytochrome c_{M552} [b]	Periplasmic cytochrome c_{554} or hydroxylamine oxidoreductase, transient interaction, *Nitrosomonas europaea*	[55,56]
	Sqr		
	No predicted TMS	Membrane-attached sulfide:(ubi)quinone oxidoreductase (FAD) that forms polysulfide in the periplasm, *Rhodobacter capsulatus.*	[57]
	Gdh/Asd		
	No predicted TMS	Periplasmic soluble glucose/aldose sugar dehydrogenase (PQQ) predicted to be part of a periplasmic electron transfer system feeding electrons into the ubiquinone pool. *E. coli.*	[58]
Type ③	SdhC/SdhD		
	3 TMS each (His: 1-1-0 in both SdhC and SdhD)	SdhAB (FAD, Fe/S), formation of membrane-bound succinate:(methyl-) menaquinone dehydrogenase complex (Type A SQO with 2 haem *b* [59]), *Thermoplasma acidophilum*, *Natronobacterium pharaonis*, *Halobacterium salinarum*	[60]
	SdhC/SdhD		
	3 TMS each (His: 0-1-0 in both SdhC and SdhD)	SdhAB (FAD, Fe/S), formation of membrane-bound succinate:ubiquinone dehydrogenase complex (Type C SQO with 1 haem *b* [59]), eukaryotic mitochondria and bacteria like *E. coli* and *P. denitrificans*	[42,61]
	SdhE/SdhF	SdhAB (FAD, Fe/S), formation of "non-classical" membrane-bound succinate:caldariella quinone dehydrogenase complex (Type E SQO [59]), *Acidianus ambivalens*, *Sulfolobus acidocaldarius*. SdhE contains a characteristic "CCG motif" comprising ten conserved cysteine residues. Note that SdhE and SdhF are often erroneously designated SdhC and SdhD in data bases.	[60]
	DoxD/DoxA		
	4 TMS in DoxS, 1 TMS in DoxA	Membrane-bound tetrathionate-forming thiosulfate:quinone (oxido) reductase (TQO). The purified enzyme contains caldariella quinone and sulfolobus quinone (CQ-6 and SQ-6) and probably forms a short electron transport chain with a CQ:O_2 oxidoreductase. *Acidianus ambivalens*. Cytoplasmic thiosulfate oxidation is likely. The *doxDA* genes are fused in *Acidithiobacillus ferrooxidans* and *Bacteroides* species.	[62]
	GlpC		
	No predicted TMS	GlpAB (FAD/FMN), anaerobic *sn*-glycerol-3-phosphate dehydrogenase. GlpC contains two cysteine clusters typical for Fe/S binding and two "CCG" signatures. *E. coli*	[63]
	Ndh		
	No predicted TMS	Single subunit non-electrogenic (alternative) NADH:ubiquinone oxidoreductase (Type II Ndh, Ndh-2) (FAD). *E. coli*	[48]
	GlpD, PoxB, Dld (LctD), DadA		
	No predicted TMS	Aerobic *sn*-glycerol-3-phosphate dehydrogenase; pyruvate oxidase (FAD), aerobic lactate dehydrogenase; D-amino acid dehydrogenase. Single membrane-associated but mostly hydrophilic proteins. *E. coli*	[63]
	ETF:ubiquinone oxidoreductase (ETF–QO)	Acyl CoA dehydrogenase (FAD) and ETF (electron-transferring flavoprotein) involved in fatty acid oxidation (FAD, Fe/S)	[47]
Type ④	SdhC		
	5 TMS (His: 1-1-1-1-0)	SdhAB (FAD, Fe/S), formation of membrane-bound succinate:menaquinone dehydrogenase complex (Type B SQO with 2 haem *b* [59]). *Bacillus licheniformis*, *B. subtilis* and other gram-positive bacteria as well as non-fumarate-respiring sulfate reducers like *Desulfovibrio vulgaris*.	[8,64]
Type ⑤		No examples given	
Type ⑥	NapC[b]	NapAB (Mo, Fe/S, haem *c*), transient electron transfer to periplasmic nitrate reductase.	[7]
	NrfH[b]	NrfA (haem *c*), formation of membrane-bound cytochrome *c* nitrite reductase complex (NrfHA), *W. succinogenes* and *Desulfovibrio vulgaris*.	[7,24,26,65]
	NirT[b]	NirS (haems *c* and d_1), periplasmic NO-producing nitrite reductase, *Pseudomonas stutzeri.*	[66]
	CymA[b]	Various periplasmic oxidoreductases, *Shewanella* spp.	[39]
	TorC/DorC[b]	TorA/DorA (Mo, Fe/S), periplasmic TMAO and/or DMSO reductase.	[65,67]
	NapH[c]	NapG (Fe/S), menaquinol dehydrogenase in periplasmic Nap system of *W. succinogenes*. Ubiquinol dehydrogenase in *E. coli*.	[34–36]
	NosH[c]	NosG (Fe/S), possibly involved in electron transfer to periplasmic cytochrome *c* N_2O reductase, *W. succinogenes*.	[35,38]
	NrfD[d]	NrfABC (Haem *c*, Fe/S), electron transport to periplasmic cytochrome *c* nitrite reductase NrfA, *E. coli*.	[23,68]
	MccD[d]	MccAC (Haem *c*, Fe/S). Hypothetical electron transport to/from the octahaem cytochrome *c* MccA, *W. succinogenes*.	[69]
	PsrC[d]	PsrAB (Mo, Fe/S), formation of membrane-bound polysulfide reductase complex, *W. succinogenes*, *Thermus thermophilus*. This type of enzyme may also catalyse thiosulfate and/or tetrathionate reduction.	[28,29]
	TtrC[d]	TtrAB (Mo, Fe/S), formation of a membrane-bound quinol:tetrathionate reductase complex (producing thiosulfate), *Salmonella* spp.	[30]
	DmsC		
	8 TMS	DmsAB (Mo, Fe/S), formation of a membrane-bound menaquinol:dimethylsulfoxide reductase complex, *E. coli*.	[70]
Type ⑦	FrdC/FrdD		
	3 TMS each	FrdAB (FAD, Fe/S), formation of membrane-bound menaquinol:fumarate reductase complex (Type D SQO lacking haem [59]), *E. coli*.	[42,71]

Table 1 *(continued)*

Enzyme class	Quinone- or MP-reactive protein (subunit) and mode of membrane interaction[a]	Redox partner (cofactors), mode of interaction, representative organism(s) and enzyme (complex) designation[a]	References
Type ⑧	FrdC 5 TMS (His: 1-1-1-1-0)	FrdAB (FAD, FeS), formation of membrane-bound menaquinol:fumarate reductase complex (Type B SQO with 2 haem *b* [59]). *W. succinogenes* and other ε-proteobacteria, *D. desulfuricans*.	[43–46]
Type ⑨	NarI 5 TMS (His: 0-2_9-0-0-2_{17})	NarGH (Mo, Fe/S), formation of membrane-bound nitrate reductase complex. *E. coli*.	[9,11]
	HdrE 5 TMS (His: 0-1-0-0-2_{18})	HdrD (Fe/S), formation of membrane bound MPH_2:heterodisulfide reductase complex, *Methanosarcina* spp.	[15,72,73]
	HmeC 5 TMS (His: 0-2_9-0-0-2_{17})	Hme complex (4 or 5 proteins) (Haem *c*, Fe/S); putative formation of a menaquinone-reactive membrane bound oxidoreductase complex. *Archaeoglobus* spp.	[17,18]
	CytAB 7 and 8 TMS, respectively	Cytochrome *bd* ubiquinol oxidase. Note that the two haem *b* groups are bound by different polypeptides. *E. coli*.	[74]

Enzymes (or enzyme complexes) are classified on the basis of their quinone- (or methanophenazine-) reactive subunits. Some of these proteins form stable membrane-bound complexes in conjunction with appropriate redox partner proteins while others transfer electrons only during transient protein–protein interactions, thereby enabling the formation of electron transfer networks.

[a] Common designations of homologous proteins are given in parentheses. Transmembrane protein segments (TMS) were predicted using the TMpred program. A code illustrates the localisation of conserved histidine residues in TMS that are either known or likely axial haem *b* ligands, e.g. "His: 1-1-0-2_{13}" means that one such histidine is located in TMS 1, one in TMS 2 and two (separated by 13 other residues) in TMS 4. Fe/S refers to the presence of one or more iron sulfur centres. Mo denotes the presence of a molybdenum cofactor (usually Mo-*bis*-MGD).

[b] Member of the NapC/NrfH family. These proteins usually contain 4 haem *c* groups and are membrane-anchored via one N-terminal TMS. In addition, the TorC/DorC proteins possess a C-terminally fused monohaem cytochrome *c* domain.

[c] NapH/NosH proteins contain 4 TMS, 2 conserved CX_3CP motifs and are predicted to possess two [4Fe/4S] clusters of unknown function located at the N side of the membrane.

[d] Member of the NrfD/PsrC family forming 8 or, in case of TtrC, 9 TMS.

observations and hypotheses on the mechanism of proton translocation drawn from biophysical experiments with cell sphaeroplasts [10,11]. NarA consists of the structural components NarG, NarH and NarI. The nitrate-reducing subunit NarG binds a molybdenum ion as part of a Mo-*bis*-molybdopterin guanine dinucleotide cofactor (Mo-*bis*-MGD) and an iron sulfur cluster. NarH binds four iron sulfur clusters and mediates electron transfer to NarG. NarG and NarH constitute the reductase module that receives electrons from NarI, an integral membrane protein that binds two *b*-type haems and draws electrons out of the menaquinol pool to NarH. NarG and NarH are located at the N face of the cytoplasmic membrane. In NarI one of the two haems is located at the P side of the protein and the other is at the N side. NarI receives electrons from menaquinol at the P side and electrons move down a ~9 nm wire of 8 redox centres and ultimately reduce nitrate at the N side of the cytoplasmic membrane (Fig. 1C). This structurally defined nanowire comprises two haems, five iron sulfur clusters and the Mo-*bis*-MGD [11]. Since oxidation of menaquinol occurs at the periplasmic side of NarI, the protons are released into the periplasm and the 2 electrons are moved from the haem on the P side to the haem on the N side which has a more positive E_0'. The transmembrane charge separation makes the Nar enzyme electrogenic (or proton motive) in that a net of 2 positive charges is translocated across the membrane during transfer of 2 electrons to nitrate ($H^+/e^- = 1$), thus transducing the free energy in the QH_2/nitrate couple (~420 mV) into a *pmf* via an electrogenic redox half loop mechanism at a thermodynamic efficiency of about 50%. Readers should be aware that this value is based on the standard midpoint potentials of the redox couples involved in the overall reactions. The true efficiencies rely on the working redox potentials of electron donor and acceptor couples which in turn depend on the local steady state concentrations of the reactants in the vicinity of the enzymes' active sites

The NarGHI redox loop serves to oxidise menaquinol to menaquinone. In order to sustain turnover of NarGHI, it must be coupled to a donor:menaquinone dehydrogenase to replenish menaquinol. *E. coli* produces formate under anaerobic conditions when organic carbon substrates are catabolised via pyruvate and the enzyme pyruvate formate lyase. When nitrate reduction by NarA is coupled to electron input from formate via the nitrate-inducible formate dehydrogenase (Fdh-N), two redox half loops are brought together and the FdhN-NarA respiratory chain of *E. coli* emerges as paradigm for a full proton motive redox loop (Fig. 1C). In Fdh-N, by contrast to NarGHI, the catalytic site is at the P side of the membrane. However, like NarA, the electrons generated pass down a ~9 nm wire of redox centres that in this case connect the Mo-*bis*-MGD cofactor of Fdh-N, located in the periplasm, to a menaquinone reductase site at the N face of the cytoplasmic membrane. Like in NarGHI, the wire comprises five iron sulfur clusters and two *b*-type haems. A large potential drop (~340 mV) from formate to menaquinone allows efficient electron transfer against the *pmf* of ~200 mV and the whole process serves, like NarA, to effectively translocate 2 positive charges across the membrane for every two electrons extracted from formate ($H^+/e^- = 1$). Together then, the electron-carrying arms of Fdh-N and NarA form a proton motive redox loop that spans an electron-transfer distance of some 15 nm, has a $\Delta E_0'$ of 840 mV (−420 mV to +420 mV) and a coupling stoichiometry of $4H^+/2e^-$(~50% thermodynamic efficiency). When the whole electron transfer ladder from the Fdh-N Mo-*bis*-MGD via the quinone pool to the Nar Mo-*bis*-MGD is considered, it should be noted that the intermediary haem and iron sulfur cluster electron carriers are one-electron transfer centres. However, as formate oxidation, quinone reduction, quinol oxidation and nitrate reduction are two-electron reactions, the Mo-*bis*-MGD cofactors and Q/QH_2 binding sites at either end of the two intramolecular nanowires are crucial for coupling the one- and two-electron oxidoreductions. Generally, if two redox cofactors are positioned within ~1.4 nm or less of each other, rapid electron transfer will take place provided there is a sufficiently strong overall thermodynamic driving force [12]. In the case of the formate/menaquinol and menaquinol/nitrate redox couples there is such a driving force and thus electrons will move rapidly through the wire being 'pushed' by formate and 'pulled' by nitrate. The involved 'wire-like' arrangement of the electron transferring cofactors can be seen in a number of different kinds of respiratory enzymes, for example in the membrane-bound Ni/Fe-hydrogenase (H_2:quinone dehydrogenase) which is predicted to operate a redox half loop mechanism similar to Fdh-N (type ① in Table 1). Recently, a novel group of archaeal and bacterial enzymes emerged where a NarG-like protein was found to contain a typical twin-arginine signal peptide suggesting an outside-orientation of the catalytic subunit [13,14]. The bioenergetic significance of such enzymes is not known as the corresponding genes are not accompanied by a *narI*-type gene.

Another, but less well known, example of mechanism A is the H_2: heterodisulfide oxidoreductase system used in mixed disulfide

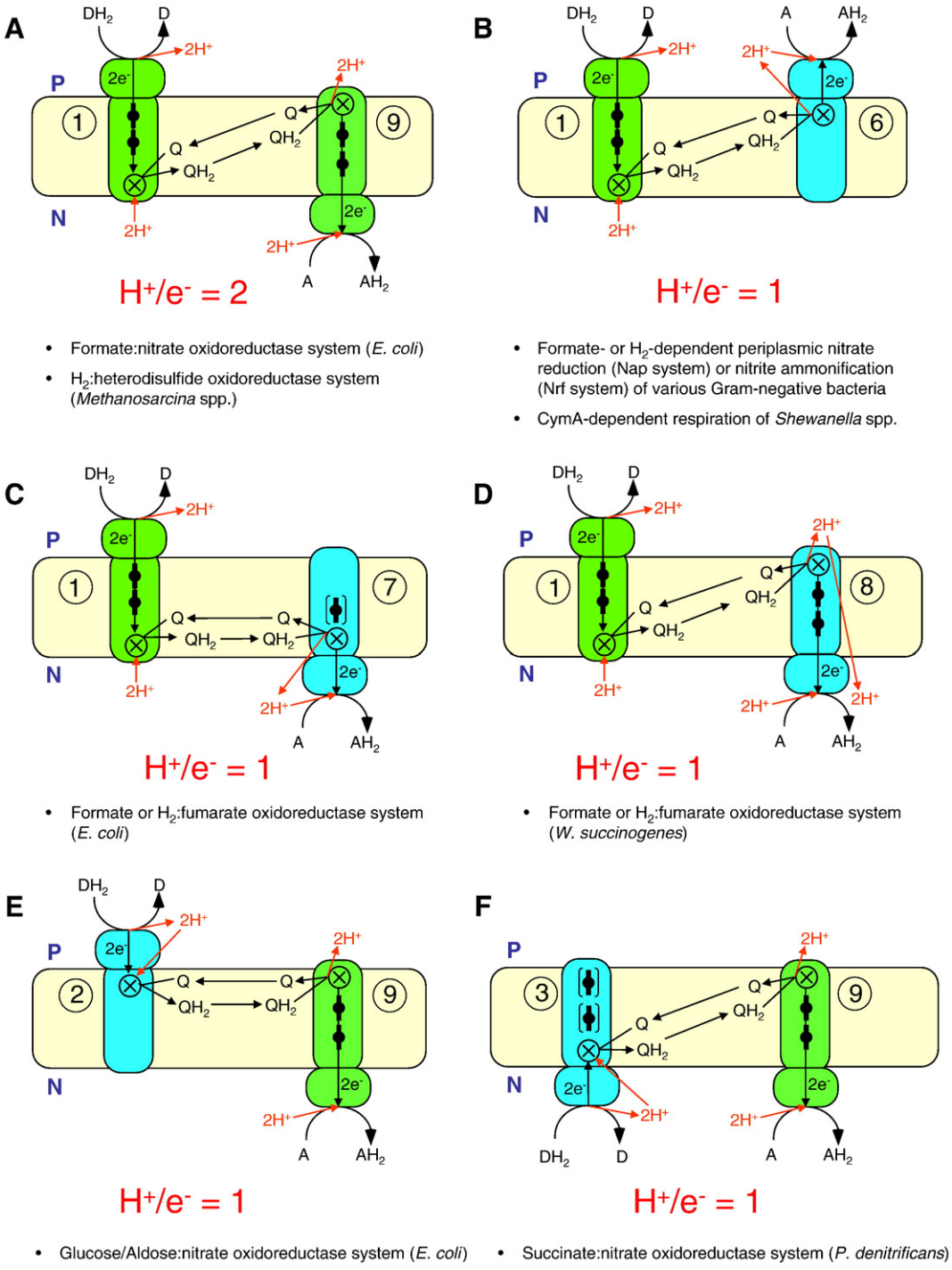

Fig. 3. Combination of donor:quinone dehydogenases and quinol:acceptor reductases in simple quinone-dependent respiratory chains. The enzyme classification presented in Fig. 2 allows for six possible arrangements that raise a *pmf* by quinone-mediated redox reactions (mechanisms A–F). Model A represents the full proton motive redox loop (*cf.* Fig. 1B). Examples of redox systems for which biochemical evidence is available are stated. See text for details.

respiration of methanogenic *Methanosarcina* species [15]. This system consists of two methanophenazine-reactive enzyme complexes, namely F_{420}-nonreducing Ni/Fe-hydrogenase (type ①) and dihydro-methanophenazine-reactive heterodisulfide reductase (type ⑨). Both enzymes contain membrane anchor subunits (VhoC and HdrE) with 5 transmembrane segments (TMS) each that are likely to bind two haem *b* groups to ensure transmembrane electron delivery (Table 1). Only three histidines are conserved in HdrE, but there is also a conserved methionine in the second TMS, hence it is conceivable that HdrE binds two haems (one each with His–His and His–Met ligation) stacked across the membrane. This would make HdrE operationally equivalent to NarI but conducting electrons from a dihydro-methanophenazine oxidation site located at the P side to a heterodisulfide reductase site located at the N side of the membrane. Heterodisulfide reduction by H_2 has been shown to be coupled with the translocation of 3–4 $H^+/2e^-$ in *Methanosarcina mazei* Gö1 which is in accordance with mechanism A [16]. Both electron carrying arms of the redox loop have been investigated separately using inverted cell vesicles and 2-hydroxy-phenazine/dihydro-2-hydroxyphenazine as redox mediator demonstrating that both reactions are electrogenic [16]. However, the precise

localisation and architecture of the MP/MPH_2-reactive sites remain to be established. A predicted di-haem cytochrome *b* (HmeC, type ⑨ in Table 1) which is similar to HdrE was proposed as the membrane anchor of a menaquinol:acceptor dehydrogenase Hme complex in the sulfate-reducing archaea *Archaeoglobus fulgidus* and *Archaeoglobus profundus* that might play a role in disulfide reduction or a related process [17,18]. The precise haem *b* content of HmeC was not reported but four conserved histidine residues are present (Table 1). Similar to HmeC is DsrM, a potential di-haem cytochrome *b* found in *Desulfovibrio* species, *Chlorobium tepidum*, *Allochromatium vinosum* and *Desulfitobacterium hafniense* (occasionally the corresponding gene is part of a gene cluster encoding sulfite reductase) [19]. In fact, a variety of such proteins from sulfate reducers (named DsrM, TmcC, HmcE, HmcC and QmoC) have been characterised recently [20–22]. The proteins all represent putative subunits of membrane-bound and possibly menaquinone/menaquinol-reactive complexes whose detailed function in substrate conversion and bioenergetics is yet unresolved. Depending on their substrate (possibly hydrogenase-reduced cytochrome c_3 or adenosine-5′-phosphosulfate/sulfite), such complexes have to be classified as either type ① or ⑨.

3.2. Mechanism B

This mode of operation is arguably the most common mechanism of those presented in this article and couples the electrogenic donor: quinone dehydrogenase of type ① to an electroneutral reaction involving a quinol:acceptor reductase system of type ⑥ (Fig. 3B and Table 1). Type ⑥ systems contain some of the most widespread bacterial quinol dehydrogenase modules, namely those belonging to the NapC/NrfH, NapH and NrfD/PsrC families [7]. Although the free energy of the quinol:acceptor couple (which in some cases is high enough to drive proton translocation) is not transduced in *pmf* formation, enzymes of type ⑥ play an essential role in redox balancing by supplying quinone for the electrogenic type ① enzyme.

A prominent example of mechanism B is the formate- (or H_2-) dependent periplasmic nitrate ammonification pathway of enteric bacteria and other species like *Desulfovibrio desulfuricans* and *W. succinogenes* that couple quinol oxidation to nitrate reduction, thus producing nitrite which is further reduced to ammonium (note that some species contain only one of these systems, e.g. many nitrate-denitrifying bacteria or sulfate reducers that cannot convert nitrate but reduce nitrite to ammonium) [23]. The quinol pool is oxidised by the Nap and Nrf systems in order to catalyse electroneutral nitrate reduction to nitrite and nitrite reduction to ammonium, respectively. Consequently, the whole respiratory nitrate ammonification system relies on *pmf* generation at the level of electron input into the Q pool (for example via a type ① enzyme), rather than at the level of electron output. Hence, the role of the Nap and Nrf systems is to serve turnover of the QH_2-pool to ensure a continued supply of oxidised quinone for the quinone-reducing electron input components. Experimental support for mechanism B comes from experiments performed with *W. succinogenes* demonstrating electrogenic electron transport from formate to nitrite in liposomes containing formate dehydrogenase (FdhABC, type ①), menaquinone and the cytochrome *c* nitrite reductase complex (NrfHA, type ⑥) [24]. The NrfH protein was shown to be required for menaquinol oxidation [24,25] and this view was supported by the crystal structure of the *D. vulgaris* NrfHA complex [26]. In contrast to the functionally similar NapC proteins (Table 1), NrfH forms a stable complex with its redox partner, the nitrite-reducing NrfA. In this case, complex formation is apparently facilitated by the fact that a NrfA lysine residue acts as an axial iron ligand of one of the NrfH haem *c* groups [26]. Interestingly, NrfH is replaced by the NrfBCD system in enteric bacteria like *E. coli* [23]. Here, the membrane-bound NrfD presumably works as a menaquinol dehydrogenase that initiates electron transport via the Fe/S protein NrfC to the periplasmic pentahaem cytochrome *c* NrfB [7,27]. NrfD is the prototype of the NrfD/PsrC family that also comprises the membrane anchors of polysulfide reductase (PsrC, e.g. from *W. succinogenes* or *Thermus thermophilus*) and tetrathionate reductase (TtrC from *Salmonella enterica*) [28–30]. Such proteins form eight or nine TMS and are thought to catalyse quinol oxidation. The crystal structure of the *T. thermophilus* PsrABC complex recently demonstrated that the quinol binding site is situated at the P side of the membrane and in close distance to an iron sulfur centre of PsrB [29]. Proteoliposomes containing the PsrABC complex and either of the type ① enzyme complexes HydABC or FdhABC isolated from *W. succinogenes* membranes were found to catalyse polysulfide respiration [28]. This system, however, was dependent on the presence of methyl-menaquinone-6 (MMK-6) which is likely to be bound by PsrC. It has to be kept in mind here that the midpoint potential of the polysulfide/sulfide redox pair is more negative than that of MK/MKH_2. Consequently, the free energy change for polysulfide reduction by either H_2 or formate is not high enough to drive a H^+/e^- of 1. In fact, *W. succinogenes* polysulfide respiration was shown to be dependent on supercomplex formation between hydrogenase (or formate dehydrogenase) and polysulfide reductase and to operate at an estimated H^+/e^- of 0.5 [31]. It is conceivable that the bound MMK-6 is energetically more favourable than MK-6 to mediate electron transport between the enzyme complexes. In contrast to polysulfide respiration, MK-6 proved sufficient for the *pmf*-generating reconstitution of other respiratory chains from *W. succinogenes* in liposomes, e.g. those using nitrite and fumarate as electron acceptor, i.e. substrates with a more positive midpoint potential than that of polysulfide [24,32].

Another facet of periplasmic nitrate reduction comes from the fact that the *W. succinogenes* Nap system is independent of a NapC homologue, as opposed to other bacteria like *E. coli* [33]. It was shown recently that *W. succinogenes* relies on the NapH and NapG proteins to catalyse menaquinol oxidation and electron transport to NapA (via the essential periplasmic di-haem cytochrome *c* NapB) [34,35]. In contrast, *E. coli* apparently uses a NapGH complex to catalyse ubiquinol oxidation in order to initiate electron transport via NapC and NapB to NapA [36]. NapH forms 4 TMS across the membrane and is very likely to contain a quinol-binding site from which electrons are transferred to the periplasmic Fe/S protein NapG. Purification of a NapH-like protein has not yet been reported and no structural information is available. The two four-cysteine clusters and two Cys-X_3-Cys-Pro signatures (all conserved and predicted to be located on the N side of the membrane) have been shown to be functionally essential in *W. succinogenes* NapH [35]. NapH and NapG are encoded in many, but not all, *nap* gene clusters and NapH-like proteins (e.g. NosH, MauN, RdxA, CcoG/FixG/RdxB) are also encoded in various other genetic contexts [35,37,38]. Although unlikely, it cannot be excluded that NapH-catalysed redox reactions are coupled to proton pumping.

Interestingly, many of the quinone-reactive type ⑥ proteins discussed above do not seem to form stable membrane-bound enzyme complexes with their redox partners. In principle, this allows branched electron flow from the quinone pool to several distinct terminal reductases involved in redox balancing. Such an electron distributing role has been proposed for the NapC/NrfH family member CymA from *Shewanella* species as a *cymA* mutant was found to be defective in various modes of anaerobic respiration [39,40]. Similarly, the NrfH protein from *Campylobacter jejuni* was suggested to deliver electrons to both the Nrf and Nap systems [41].

3.3. Mechanism C

In many bacteria formate can serve as the reductant for fumarate mediated by the formate dehydrogenase complex (Fdh-N of type ①) and a quinol:fumarate reductase of type ⑦ (Fig 3C; Table 1). In this coupling mechanism the proton motive step rests with the formate

dehydrogenase which couples quinone reduction to formate oxidation in an electrogenic manner. The fumarate reductase arm of this couple is not electrogenic but serves to recycle the quinone pool so the turnover of the formate dehydrogenase is sustained. This is in line with the fact that the *E. coli* menaquinol:fumarate reductase does not contain haem *b* [42]. In many ways the function of fumarate reductase is similar to that of the periplasmic nitrate and nitrite reductases described in Mechanism B. Both are non-*pmf* generating redox cycling systems, but the key difference is the site of quinol oxidation being on the N rather than P side of the membrane in mechanism C.

3.4. Mechanism D

This mechanism is similar to mechanism C except that the quinol oxidation site of the type ⑧ quinol:acceptor reductase is located at the P side of the membrane (Fig. 3D). Therefore, the latter enzyme is a type ④ enzyme working in reverse which is expected to catalyse electrogenic quinol oxidation coupled to substrate reduction. One such enzyme is the menaquinol:fumarate reductase from ε-proteobacteria like *W. succinogenes* and *C. jejuni* (Table 1). Menaquinol oxidation by fumarate is not exergonic enough to drive *pmf* generation at a proton/electron ratio of 1. This was experimentally supported by experiments using the reconstituted electron transport chain of fumarate respiration with either formate or H_2 from *W. succinogenes* that proved that fumarate-dependent menaquinol oxidation is electroneutral [32]. The crystal structure of the corresponding menaquinol:fumarate reductase (FrdABC complex), however, clearly showed that the menaquinol oxidation site is at the P side of the membrane [43]. In order to make the FrdABC complex work in the direction of fumarate respiration, a *pmf*-consuming (uncoupling) mechanism has apparently evolved that couples the redox reaction to proton translocation from the P to the N side. This mechanism, the so-called 'E-pathway' [44], was confirmed experimentally and it has been shown to involve the conserved residue Glu-180 of TMS 5 in FrdC (*W. succinogenes* numbering) and the ring C propionate side chain of the distal haem *b* group [45,46]. The crucial glutamate residue is absent in the di-haem cytochrome *b* membrane anchor subunits of type ④ succinate:menaquinone dehydrogenases.

3.5. Mechanism E

In this case an electroneutral type ② donor:quinone dehydrogenase is coupled to an electrogenic quinol:acceptor reductase of type ⑨ (Fig. 3E). Examples where conceptually such a coupling could take place would be the periplasmic oxidation of sugars by PQQ-dependent glucose/aldose sugar dehydrogenases (Table 1). Membrane-bound glucose dehydrogenases, for example Gdh from *E. coli*, are quinone-dependent enzymes that oxidise glucose to gluconolactone using ubiquinone (UQ) as electron acceptor. The enzyme does not have any integral membrane redox centres and so the UQ is reduced at the periplasmic face of the membrane rendering Gdh an electroneutral type ② donor:quinone dehydrogenase. At low oxygen tensions in the presence of nitrate an outlet for recycling the UQ-pool is NarGHI, the prototype electrogenic quinol:acceptor reductase of type ⑨. In principal, the type ② sulfide:quinone dehydrogenase could also be coupled to a type ⑨ quinol:acceptor reductase, although this has not yet been shown experimentally.

3.6. Mechanism F

In this case an electroneutral type ③ donor:quinone dehydrogenase is coupled to an electrogenic quinol:acceptor reductase of type ⑨ (Fig. 3F). This pairing is quite common in anaerobic nitrate-dependent metabolism as substrates that feed the citric acid cycle would pass electrons to nitrate reductase (type ⑨) via succinate dehydrogenase (type ③) in, for example, *P. denitrificans* or *E. coli* (Table 1). Since the structures of the succinate:quinone oxidoreductase and membrane-bound nitrate reductase of *E. coli* have been solved, a full structural resolution of a mechanism F coupling is available. Organic electron donors that feed into metabolism via the β-oxidation of fatty acids also illustrate an example of an electroneutral type ③ donor:quinone dehydrogenase called electron transfer flavoprotein (ETF) quinone oxidoreductase that couples fatty acid oxidation to the respiratory chain. A structure of this enzyme from porcine mitochondria has recently emerged that has many bacterial homologues [47]. Also, in many bacteria, in addition to the proton-pumping NADH dehydrogenase (complex I), a second type of NADH dehydrogenase (Ndh-2; Table 1) is assembled that is non-proton pumping and is thus another widespread example of an electroneutral type ③ donor:quinone dehydrogenase [48].

4. Conclusions and outlook

Although the structures of many oxidoreductases that underpin microbial respiratory diversity are known, for example many of the N-cycling oxidoreductases, the electron transport chains that mediate electron transfer between the quinone/quinol pool to or from these termini are less well resolved. In some cases these electron input or egress systems are tightly associated with the terminal oxidoreductase and here studies are frequently more advanced (see Mechanism A in section 3). However, in many cases the quinone/quinol-reactive modules do not tightly associate with the terminal oxidoreductase and here purification and characterisation is often more difficult owing to lack of convenient assays. These systems represent challenges for the future.

A feature of many of the stable complexes is the presence of di-haem cytochrome *b* membrane anchors that are critical to transmembrane charge separation in a range of biochemically unrelated systems. This conservation of the mechanism for transmembrane electron transfer also appears to be associated with a preference for electron transfer to iron sulfur clusters that can be either [2Fe/2S], [3Fe/4S] or [4Fe/4S]. The formation of a tight complex prevents the possibility of promiscuous electron transfer. Interestingly, enzymes of types ①, ④ and ⑨ (where H^+/e^- is either +1 or −1) usually form stable membrane-bound complexes whereas many of the proteins involved in electroneutrally operating enzyme systems ($H^+/e^-=0$) apparently interact only transiently, a fact that might accelerate electron transfer in redox balancing reactions. When a quinone/quinol-reactive module is not tightly associated with a terminal oxidoreductase, promiscuity becomes a possibility if multiple redox partners are available. This is clearly apparent in many bacterial systems and a prominent example is the tetrahaem *c*-type cytochrome CymA that has been shown to be involved in electron transfer to a wide range of systems (see above). CymA belongs to the NapC/NrfH family of multihaem *c*-type cytochromes that is widespread amongst bacterial species and appears to represent a frequently used evolutionary solution for a quinol:cytochrome *c* reductase. However, apart from the cytochrome bc_1 complex, no quinol:cytochrome *c* reductase has yet emerged that is intrinsically energy transducing. This observation is in marked contrast to the many different types of quinol:cytochrome *b* dehydrogenases presented in this article. Another observation that emerges from consideration of the quinol-dependent electron transfer systems explored here is the flexibility for coupling *pmf* generation to donor oxidation, acceptor reduction or both. This can serve to optimise metabolism according to the nature of the donors and acceptors available. It also provides for metabolic options for the organisms that are not only living with two substrates in nature, but employ electron transfer networks that jointly contribute to *pmf* generation.

Many exergonic reactions have been recognised in recent years to sustain microbial growth, predominantly in anaerobic environments. Examples are anaerobic ammonium oxidation using nitrite as electron

acceptor (the anammox process) or nitrite-dependent anaerobic oxidation of methane [49,50]. In these cases, it is likely that metabolic principles will be initially hypothesised on the basis of genome sequences. We hope that the classification of enzymes and *pmf*-generating mechanisms discussed here will be useful in defining and reconstructing electron transport networks from genetic data where biochemical characterisation is lacking. Undoubtedly, many more as yet unknown enzymes fitting into the framework presented here will be discovered in the future. All of these will rely on general thermodynamic principles and most of them will depend on known electron transport mediators and cofactors. We hope that the *pmf*-generating redox loop mechanisms presented here will be used as a guideline for the future characterisation of structural, enzymatic and bioenergetic aspects of quinone/quinol-reactive proteins.

Acknowledgements

The authors are grateful to their co-workers of cited publications for their contributions to various aspects of respiratory electron transport chains. We thank Reiner Hedderich (Marburg), C. Roy D. Lancaster (Homburg/Saar), Ben Berks and Stuart Ferguson (both Oxford) for valuable discussions and Andrew Gates (Norwich) for the preparation of Fig. 1C. Work in the authors' labs was supported by the Deutsche Forschungsgemeinschaft (DFG) and the Biotechnology and Biological Sciences Research Council (BBSRC). JS is grateful to the DFG for awarding a Heisenberg Fellowship; DJR thanks the Royal Society and Wolfson Foundation for a Merit Award.

References

[1] D.G. Nicholls, S.J. Ferguson, Bioenergetics 3, Academic Press, Amsterdam, 2002.
[2] P. Mitchell, Chemiosmotic Coupling in Oxidative and Photosynthetic Phosphorylation, Glynn Research, Bodmin, 1966.
[3] P. Mitchell, Chemiosmotic Coupling and Energy Transduction, Glynn Research, Bodmin, 1968.
[4] P. Mitchell, Coupling of phosphorylation to electron and hydrogen transfer by a chemiosmotic type of mechanism, Nature 191 (1961) 144–148.
[5] D.J. Richardson, Bacterial respiration: a flexible process for a changing environment, Microbiology 146 (2000) 551–571.
[6] P. Mitchell, Keilin's respiratory chain concept and its chemiosmotic consequences, Science 206 (1979) 1148–1159.
[7] J. Simon, M. Kern, Quinone-reactive proteins devoid of haem *b* form widespread membrane-bound electron transport modules in bacterial anaerobic respiration, Biochem. Soc. Trans. 36 (2008) 1011–1016.
[8] T. Zaunmüller, D.J. Kelly, F.O. Glöckner, G. Unden, Succinate dehydrogenase functioning by a reverse redox loop mechanism and fumarate reductase in sulfate-reducing bacteria, Microbiology 152 (2006) 2443–2453.
[9] D. Richardson, G. Sawers, PMF through the redox loop, Science 295 (2002) 1842–1843.
[10] R.W. Jones, A. Lamont, P.B. Garland, The mechanism of proton translocation driven by the respiratory nitrate reductase complex of *Escherichia coli*, Biochem. J. 190 (1980) 79–94.
[11] M.G. Bertero, R.A. Rothery, M. Palak, C. Hou, D. Lim, F. Blasco, J.H. Weiner, N.C. Strynadka, Insights into the respiratory electron transfer pathway from the structure of nitrate reductase A, Nature Struct. Biol. 10 (2003) 681–687.
[12] C.C. Page, C.C. Moser, X. Chen, P.L. Dutton, Natural engineering principles of electron tunnelling in biological oxidation-reduction, Nature 402 (1999) 47–52.
[13] R.M. Martinez-Espinosa, E.J. Bridge, M.J. Bonete, J.N. Butt, C.S. Butler, F. Sargent, D.J. Richardson, Look on the positive side! The orientation, identification and bioenergetics of "archaeal" membrane-bound nitrate reductases, FEMS Microbiol. Lett. 276 (2007) 129–139.
[14] F. Sargent, Constructing the wonders of the bacterial world: biosynthesis of complex enzymes, Microbiology 153 (2007) 633–651.
[15] U. Deppenmeier, V. Müller, Life close to the thermodynamic limit: how methanogenic archaea conserve energy, Results Probl. Cell. Differ. 45 (2007) 123–152.
[16] T. Ide, S. Bäumer, U. Deppenmeier, Energy conservation by the H_2:heterodisulfide oxidoreductase from *Methanosarcina mazei* Gö1: identification of two proton-translocating segments, J. Bact. 181 (1999) 4076–4080.
[17] G.J. Mander, E.C. Duin, D. Linder, K.O. Stetter, R. Hedderich, Purification and characterization of a membrane-bound enzyme complex from the sulfate-reducing archaeon *Archaeoglobus fulgidus* related to heterodisulfide reductase from methanogenic archaea, Eur. J. Biochem. 269 (2002) 1895–1904.
[18] G.J. Mander, A.J. Pierik, H. Huber, R. Hedderich, Two distinct heterodisulfide reductase-like enzymes in the sulfate-reducing archaeon *Archaeoglobus profundus*, Eur. J. Biochem. 271 (2004) 1106–1116.
[19] R.H. Pires, S.S. Venceslau, F. Morais, M. Teixeira, A.V. Xavier, I.A.C. Pereira, Characterization of the *Desulfovibrio desulfuricans* ATCC 27774 DsrMKJOP complex — a membrane-bound redox complex involved in the sulfate respiratory pathway, Biochemistry 45 (2006) 249–262.
[20] P.M. Matias, I.A.C. Pereira, C.M. Soares, M.A. Carrondo, Sulphate respiration from hydrogen in *Desulfovibrio* bacteria: a structural biology overview, Prog. Biophys. Mol. Biol. 89 (2005) 292–329.
[21] R.H. Pires, A.I. Lourenço, F. Morais, M. Teixeira, A.V. Xavier, L.M. Saraiva, I.A.C. Pereira, A novel membrane-bound respiratory complex from *Desulfovibrio desulfuricans* ATCC 27774, Biochim. Biophys. Acta 1605 (2003) 67–82.
[22] P.M. Pereira, M. Teixeira, A.V. Xavier, R.P. Louro, I.A.C. Pereira, The Tmc complex from *Desulfovibrio vulgaris* Hildenborough is involved in transmembrane electron transfer from periplasmic hydrogen oxidation, Biochemistry 45 (2006) 10359–10367.
[23] J. Simon, Enzymology and bioenergetics of respiratory nitrite ammonification, FEMS Microbiol. Rev. 26 (2002) 285–309.
[24] J. Simon, R. Gross, O. Einsle, P.M.H. Kroneck, A. Kröger, O. Klimmek, A NapC/NirT-type cytochrome *c* (NrfH) is the mediator between the quinone pool and the cytochrome *c* nitrite reductase of *Wolinella succinogenes*, Mol. Microbiol. 35 (2000) 686–696.
[25] M. Kern, O. Einsle, J. Simon, Variants of the tetrahaem cytochrome *c* quinol dehydrogenase NrfH characterize the menaquinol binding site, the haem *c* binding motifs and the transmembrane segment, Biochem. J. 414 (2008) 73–79.
[26] M.L. Rodrigues, T.F. Oliveira, I.A.C. Pereira, M. Archer, X-ray structure of the membrane-bound cytochrome *c* quinol dehydrogenase NrfH reveals novel haem coordination, EMBO J. 25 (2006) 5951–5960.
[27] T.A. Clarke, J.A. Cole, D.J. Richardson, A.M. Hemmings, The crystal structure of the pentahaem *c*-type cytochrome NrfB and characterization of its solution-state interaction with the pentahaem nitrite reductase NrfA, Biochem. J. 406 (2007) 19–30.
[28] W. Dietrich, O. Klimmek, The function of methyl-menaquinone-6 and polysulfide reductase membrane anchor (PsrC) in polysulfide respiration of *Wolinella succinogenes*, Eur. J. Biochem. 269 (2002) 1086–1095.
[29] M. Jormakka, K. Yokoyama, T. Yano, M. Tamakoshi, S. Akimoto, T. Shimamura, P. Curmi, S. Iwata, Molecular mechanism of energy conservation in polysulfide respiration, Nat. Struct. Mol. Biol. 15 (2008) 730–737.
[30] M. Hensel, A.P. Hinsley, T. Nikolaus, G. Sawers, B.C. Berks, The genetic basis of tetrathionate respiration in *Salmonella typhimurium*, Mol. Microbiol. 32 (1999) 275–288.
[31] O. Klimmek, W. Dietrich, F. Dancea, Y.-J. Lin, S. Pfeiffer, F. Löhr, H. Rüterjans, R. Gross, J. Simon, A. Kröger, Sulfur respiration, in: D. Zannoni (Ed.), Respiration in Archaea and Bacteria, Vol. 2, Springer, Dordrecht, 2004, pp. 217–232, Diversity of prokaryotic respiratory systems.
[32] S. Biel, J. Simon, R. Gross, T. Ruiz, M. Ruitenberg, A. Kröger, Reconstitution of coupled fumarate respiration in liposomes by incorporating the electron transport enzymes isolated from *Wolinella succinogenes*, Eur. J. Biochem. 269 (2002) 1974–1983.
[33] J. Simon, M. Sänger, S.C. Schuster, R. Gross, Electron transport to periplasmic nitrate reductase (NapA) of *Wolinella succinogenes* is independent of a NapC protein, Mol. Microbiol. 49 (2003) 69–79.
[34] M. Kern, A.M. Mager, J. Simon, Role of individual *nap* gene cluster products in NapC-independent nitrate respiration of *Wolinella succinogenes*, Microbiology 153 (2007) 3739–3747.
[35] M. Kern, J. Simon, Characterization of the NapGH quinol dehydrogenase complex involved in *Wolinella succinogenes* nitrate respiration, Mol. Microbiol. 69 (2008) 1137–1152.
[36] T.H.C. Brondijk, A. Nilavongse, N. Filenko, D.J. Richardson, J.A. Cole, NapGH components of the periplasmic nitrate reductase of *Escherichia coli* K-12: location, topology and physiological roles in quinol oxidation and redox balancing, Biochem. J. 379 (2004) 47–55.
[37] B.C. Berks, M.D. Page, D.J. Richardson, A. Reilly, A. Cavill, F. Outen, S.J. Ferguson, Sequence analysis of subunits of the membrane-bound nitrate reductase from a denitrifying bacterium: the integral membrane subunit provides a prototype for the dihaem electron-carrying arm of a redox loop, Mol. Microbiol. 15 (1995) 319–331.
[38] J. Simon, O. Einsle, P.M.H. Kroneck, W.G. Zumft, The unprecedented *nos* gene cluster of *Wolinella succinogenes* encodes a novel respiratory electron transfer pathway to cytochrome *c* nitrous oxide reductase, FEBS Lett. 569 (2004) 7–12.
[39] J.M. Myers, C.R. Myers, Role of the tetraheme cytochrome CymA in anaerobic electron transport in cells of *Shewanella putrefaciens* MR-1 with normal levels of menaquinone, J. Bacteriol. 182 (2000) 67–75.
[40] C. Schwalb, S.K. Chapman, G.A. Reid, The tetraheme cytochrome CymA is required for anaerobic respiration with dimethyl sulfoxide and nitrite in *Shewanella oneidensis*, Biochemistry 42 (2003) 9491–9497.
[41] M.S. Pittman, K.T. Elvers, L. Lee, M.A. Jones, R.K. Poole, S.F. Park, D.J. Kelly, Growth of *Campylobacter jejuni* on nitrate and nitrite: electron transport to NapA and NrfA via NrfH and distinct roles for NrfA and the globin Cgb in protection against nitrosative stress, Mol. Microbiol. 63 (2007) 575–590.
[42] G. Cecchini, E. Maklashina, V. Yankovskaya, T.M. Iverson, S. Iwata, Variation in proton donor/acceptor pathways in succinate:quinone oxidoreductases, FEBS Lett. 545 (2003) 31–38.
[43] C.R.D. Lancaster, M. Auer, A. Kröger, H. Michel, Structure of fumarate reductase from *Wolinella succinogenes* at 2.2 Å resolution, Nature 402 (1999) 377–385.
[44] C.R.D. Lancaster, A.H. Haas, M.G. Madej, M. Mileni, Recent progress on obtaining theoretical and experimental support for the "E-pathway hypothesis" of coupled transmembrane electron and proton transfer in dihaem-containing quinol: fumarate reductase, Biochim. Biophys. Acta 1757 (2006) 988–995.

[45] C.R.D. Lancaster, U.S. Sauer, R. Gross, A.H. Haas, J. Graf, H. Schwalbe, W. Mäntele, J. Simon, M.G. Madej, Experimental support for the "E-pathway hypothesis" of coupled transmembrane e^- and H^+ transfer in dihemic quinol:fumarate reductase, Proc. Natl. Acad. Sci. USA 102 (2005) 18860–18865.
[46] M.G. Madej, H.R. Nasiri, N.S. Hilgendorff, H. Schwalbe, C.R.D. Lancaster, Evidence for transmembrane proton transfer in a dihaem-containing membrane protein complex, EMBO J. 25 (2006) 4963–4970.
[47] J. Zhang, F.E. Frerman, J.-J. P. Kim, Structure of electron transfer flavoprotein–ubiquinone oxidoreductase and electron transfer to the mitochondrial ubiquinone pool, Proc. Natl. Acad. Sci. USA 103 (2006) 16212–16217.
[48] S. Kerscher, S. Dröse, V. Zickermann, U. Brandt, The three families of respiratory NADH dehydrogenases, Results Prob. Cell Differ. 45 (2008) 185–222.
[49] J.G. Kuenen, Anammox bacteria: from discovery to application, Nature Rev. Microbiol. 6 (2008) 320–326.
[50] A.A. Raghoebarsing, A. Pol, K.T. van de Pas-Schoonen, A.J.P. Smolders, K.F. Ettwig, W.I.C. Rijpstra, S. Schouten, J.S. Sinninghe-Damsté, H.J.M. Op den Camp, M.S.M. Jetten, M. Strous, A microbial consortium couples anaerobic methane oxidation to denitrification, Nature 440 (2006) 918–921.
[51] A. Kröger, S. Biel, J. Simon, R. Gross, G. Unden, C.R.D. Lancaster, Fumarate respiration of *Wolinella succinogenes*: enzymology, energetics and coupling mechanism, Biochim. Biophys. Acta 1553 (2002) 23–38.
[52] M. Jormakka, B. Byrne, S. Iwata, Protonmotive force generation by a redox loop mechanism, FEBS Lett. 545 (2003) 25–30.
[53] M. Jormakka, S. Törnroth, B. Byrne, S. Iwata, Molecular basis of proton motive force generation: structure of formate dehydrogenase-N, Science 295 (2002) 1863–1868.
[54] R. Gross, R. Pisa, M. Sänger, C.R.D. Lancaster, J. Simon, Characterization of the menaquinone reduction site in the diheme cytochrome *b* membrane anchor of *Wolinella succinogenes* NiFe-hydrogenase, J. Biol. Chem. 279 (2004) 274–281.
[55] A.B. Hooper, D. Arciero, D. Bergmann, M.P. Hendrich, The oxidation of ammonia as an energy source in bacteria, in: D. Zannoni (Ed.), Respiration in Archaea and Bacteria, Vol. 2, Springer, Dordrecht, 2004, pp. 121–147, Diversity of prokaryotic respiratory systems.
[56] H.J. Kim, A. Zatsman, A.K. Upadhyay, M. Whittaker, D. Bergmann, M.K. Hendrich, A.B. Hooper, Membrane tetraheme cytochrome c_{m552} of the ammonia-oxidizing *Nitrosomonas europaea*: a ubiquinone reductase, Biochemistry 47 (2008) 6539–6551.
[57] C. Griesbeck, M. Schütz, T. Schödl, S. Bathe, L. Nausch, N. Mederer, M. Vielreicher, G. Hauska, Mechanism of sulfide-quinone reductase investigated using site-directed mutagenesis and sulfur analysis, Biochemistry 41 (2002) 11552–11565.
[58] S.M. Southall, J.J. Doel, D.J. Richardson, A. Oubrie, Soluble aldose sugar dehydrogenase from *Escherichia coli*. A highly exposed active site conferring broad substrate specificity, J. Biol. Chem. 281 (2006) 30650–30659.
[59] C.R.D. Lancaster, Succinate:quinone oxidoreductases: an overview, Biochim. Biophys. Acta 1553 (2002) 1–6.
[60] G. Schäfer, S. Anemüller, R. Moll, Archaeal complex II: "classical" and "non-classical" succinate:quinone reductases with unusual features, Biochim. Biophys. Acta 1553 (2002) 57–73.
[61] Q.M. Tran, R.A. Rothery, E. Maklashina, G. Cecchini, J.H. Weiner, *Escherichia coli* succinate dehydrogenase variant lacking the heme *b*, Proc. Natl. Acad. Sci. USA 104 (2007) 18007–18012.
[62] F.H. Müller, T.M. Bandeiras, T. Urich, M. Teixeira, C.M. Gomes, A. Kletzin, Coupling of the pathway of sulphur oxidation to dioxygen reduction: characterization of a novel membrane-bound thiosulfate:quinone oxidoreductase, Mol. Microbiol. 53 (2004) 1147–1160.
[63] G. Unden, J. Bongaerts, Alternative respiratory pathways of *Escherichia coli*: energetics and transcriptional regulation in response to electron acceptors, Biochim. Biophys. Acta 1320 (1997) 217–234.
[64] M.G. Madej, H.R. Nasiri, N.S. Hilgendorff, H. Schwalbe, G. Unden, C.R.D. Lancaster, Experimental evidence for proton motive force-dependent catalysis by the diheme-containing succinate:menaquinone oxidoreductase from the Gram-positive bacterium *Bacillus licheniformis*, Biochemistry 45 (2006) 15049–15055.
[65] R. Gross, R. Eichler, J. Simon, Site-directed modifications indicate differences in axial haem *c* iron ligation between the related NrfH and NapC families of multihaem *c*-type cytochromes, Biochem. J. 390 (2005) 689–693.
[66] A. Jüngst, S. Wakabayashi, H. Matsubara, W.G. Zumft, The *nirSTBM* region coding for cytochrome cd_1-dependent nitrite respiration of *Pseudomonas stutzeri* consists of a cluster of mono-, di-, and tetraheme proteins, FEBS Lett. 279 (1991) 205–209.
[67] S. Gon, M.-T. Giudici-Orticoni, V. Méjean, C. Iobbi-Nivol, Electron transfer and binding of the *c*-type cytochrome TorC to the trimethylamine *N*-oxide reductase in *Escherichia coli*, J. Biol. Chem. 276 (2001) 11545–11551.
[68] H. Hussain, J. Grove, L. Griffiths, S. Busby, J. Cole, A seven-gene operon essential for formate-dependent nitrite reduction to ammonia by enteric bacteria, Mol. Microbiol. 12 (1994) 153–163.
[69] R.S. Hartshorne, M. Kern, B. Meyer, T.A. Clarke, M. Karas, D.J. Richardson, J. Simon, A dedicated haem lyase is required for the maturation of a novel bacterial cytochrome *c* with unconventional covalent haem binding, Mol. Microbiol. 64 (2007) 1049–1060.
[70] R.A. Rothery, G.J. Workun, J.H. Weiner, The prokaryotic iron–sulphur molybdoenzyme family, Biochim. Biophys. Acta 1778 (2008) 1897–1929.
[71] E. Maklashina, P. Hellwig, R.A. Rothery, V. Kotlyar, Y. Sher, J.H. Weiner, G. Cecchini, Differences in protonation of ubiquinone and menaquinone in fumarate reductase from *Escherichia coli*, J. Biol. Chem. 281 (2006) 26655–26664.
[72] R. Hedderich, O. Klimmek, A. Kröger, R. Dirmeier, M. Keller, K.O. Stetter, Anaerobic respiration with elemental sulfur and with disulfides, FEMS Microbio. Rev. 22 (1999) 353–381.
[73] S. Heiden, R. Hedderich, E. Setzke, R.K. Thauer, Purification of a two-subunit cytochrome-*b*-containing heterodisulfide reductase from methanol-grown *Methanosarcina barkeri*, Eur. J. Biochem. 221 (1994) 855–861.
[74] S. Jünemann, Cytochrome *bd* terminal oxidase, Biochim. Biophys. Acta 1321 (1997) 107–127.