Hydrogenases and H+−Reduction in Primary Energy Conservation

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Hydrogenases and H^+-Reduction in Primary Energy Conservation

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Abstract Hydrogenases are metalloenzymes subdivided into two classes that contain iron-sulfur clusters and catalyze the reversible oxidation of hydrogen gas ($H_2 \leftrightarrows 2H^+ + 2\,e^-$). Two metal atoms are present at their active center: either a Ni and an Fe atom in the [NiFe]hydrogenases, or two Fe atoms in the [FeFe]hydrogenases. They are phylogenetically distinct classes of proteins. The catalytic core of [NiFe]hydrogenases is a heterodimeric protein associated with additional subunits in many of these enzymes. The catalytic core of [FeFe]hydrogenases is a domain of about 350 residues that accommodates the active site (H cluster). Many [FeFe]hydrogenases are monomeric but possess additional domains that contain redox centers, mostly Fe – S clusters. A third class of hydrogenase, characterized by a specific iron-containing cofactor and by the absence of Fe – S cluster, is found in some methanogenic archaea; this Hmd hydrogenase has catalytic properties different from those of [NiFe]- and [FeFe]hydrogenases.

The [NiFe]hydrogenases can be subdivided into four subgroups: (1) the H_2 uptake [NiFe]hydrogenases (group 1); (2) the cyanobacterial uptake hydrogenases and the cytoplasmic H_2 sensors (group 2); (3) the bidirectional cytoplasmic hydrogenases able to bind soluble cofactors (group 3); and (4) the membrane-associated, energy-converting, H_2 evolving hydrogenases (group 4). Unlike the [NiFe]hydrogenases, the [FeFe]hydrogenases form a homogeneous group and are primarily involved in H_2 evolution.

This review recapitulates the classification of hydrogenases based on phylogenetic analysis and the correlation with hydrogenase function of the different phylogenetic groupings, discusses the possible role of the [FeFe]hydrogenases in the genesis of the eukaryotic cell, and emphasizes the structural and functional relationships of hydrogenase subunits with those of complex I of the respiratory electron transport chain.

1 Introduction

Hydrogen is the most abundant element in the Universe. Initially released by abiotic processes in the Earth's early reducing atmosphere, in which it predominated (Tian et al. 2005), it has been since then a major energy source for life. The prokaryotic world has the ability to use H_2 directly, by the activity of uptake hydrogenases, or to produce H_2 directly, by the activity of H_2-evolving hydrogenases.

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The study of hydrogenase enzymes in extant microorganisms, present in particular in today's anaerobic ecosystems, may give insights into the earliest life on planet Earth. Besides, the existence of hydrogen-driven subsurface lithoautotrophic microbial ecosystems (SLIMEs), which can exist and persist independently of the products of photosynthesis (organic carbon and molecular oxygen) and probably appeared before chlorophyll-based photosynthesis was invented, may provide clues as to the nature of life in extraterrestrial worlds (Nealson et al. 2005).

If essential processes of all life, anabolic reactions via carbon and nitrogen fixation and catabolic energy metabolism via carbon oxidation and redox reaction, can be sustained by hydrogen metabolism, then the question is: how is energy conserved and converted during H_2 metabolism?

Hydrogenases catalyze the simplest chemical reaction: $2H^+ + 2\,e^- \leftrightarrows H_2$. The reaction is reversible and its direction depends on the redox potential of the components able to interact with the enzyme. In the presence of an electron acceptor, a hydrogenase will act as a H_2 uptake enzyme, while in the presence of an electron donor, the enzyme will produce H_2. About ~~720~~ a thousand hydrogenase sequences have been identified (Vignais and Billoud ~~2007~~ unpublished results), many by genome sequencing, and more than 100 have been characterized genetically and/or biochemically. By comparing their amino acid sequences, it has been possible to identify classes and subgroups of enzymes, to compare and correlate genetic, physiological and biochemical information relative to members of the subgroups, independently of their origin and their various roles in energy metabolism (Vignais et al. 2001). This review deals with the diversity of hydrogenases, their classification, and their various modes of energy conservation and conversion.

2 Diversity and Classification of Hydrogenases

Hydrogenases are generally Fe – S proteins with two metal atoms at their active site, either a Ni and an Fe atom (in [NiFe]hydrogenases) (Volbeda et al. 1995; Higuchi et al. 1997) or two Fe atoms (in [FeFe]hydrogenases) (Peters et al. 1998; Nicolet et al. 1999). A third type is the Fe – S cluster-free hydrogenase discovered in methanogenic archaea (Zirngibl et al. 1992), which functions as H_2-forming methylenetetrahydromethanopterin dehydrogenase, abbreviated Hmd. Hmd tightly binds an iron-containing light-sensitive cofactor (Lyon et al. 2004). The iron is coordinated by two CO molecules, one sulfur and a pyridone derivative linked via a phosphodiester bond to a guanosine base. The crystal structure of the apoenzyme of the Fe – S cluster-free hydrogenase has been published recently (Pilak et al. 2006). Evidence from amino acid sequences and structures indicates that the three types of hydrogenases are phylogenetically distinct classes of proteins (Vignais et al. 2001).

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2.1 The [NiFe]hydrogenases

The [NiFe]hydrogenases are the most numerous and best studied class of hydrogenases. They are found in organisms belonging to the *Bacteria* and *Archaea* domains of life. The core enzyme consists of an $\alpha\beta$ heterodimer with the large subunit (α-subunit) of ca. 60 kDa hosting the bimetallic active site and the small subunit (β-subunit) of ca. 30 kDa, the Fe – S clusters. The latter conduct electrons between the H_2-activating center and the physiological electron acceptor/donor from/to hydrogenase. Crystal structures of *Desulfovibrio* hydrogenases have shown that the two subunits interact extensively through a large contact surface and form a globular heterodimer. The bimetallic NiFe center is deeply buried in the large subunit; it is coordinated to the protein by four cysteines (Volbeda et al. 1995; Higuchi et al. 1997; Garcin et al. 1999; Matias et al. 2001). Infrared spectroscopy studies have revealed the presence of three non-protein ligands, one CO and two CN^- bound to the Fe atom (Volbeda et al. 1996; Happe et al. 1997). The [4Fe – 4S] cluster that is proximal to the active site (within 14 Å) is "essential" to H_2 activation (Volbeda et al. 1995; Fontecilla-Camps et al. 1997). Gas access to the active site is facilitated by hydrophobic channels linking the active site to the surface of the molecule (Fontecilla-Camps et al. 1997; Montet et al. 1997). Alignments of the full amino acid sequences of the small and large subunits have shown that the two subunits of [NiFe]hydrogenases evolved conjointly. That analysis led to a classification of [NiFe]hydrogenases that is consistent with the functions of the enzymes (Vignais et al. 2001).

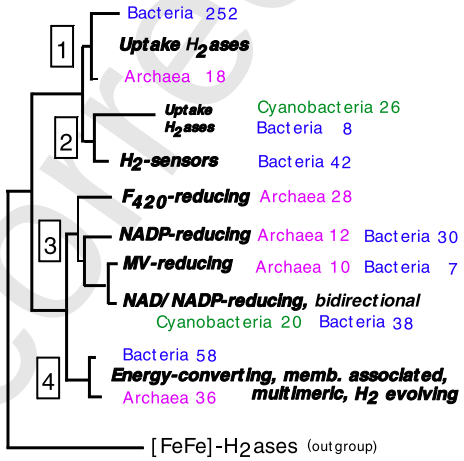

Fig. 1 Schematic representation of the phylogenetic tree of [NiFe]hydrogenases based on the complete sequences of the small and the large subunits (the same tree was obtained with each type of subunit). The number of aligned ~~sequences~~ hydrogenases is indicated (~~adapted from~~ Vignais and Billoud ~~2007~~)

originally established by Vignais et al. 2001

unpublished results

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As shown in Fig. 1, the [NiFe]hydrogenases found in *Bacteria* and in *Archaea* cluster into four groups.

2.1.1 Group 1

In Group 1 are the membrane-bound enzymes, which perform respiratory hydrogen oxidation linked to quinone reduction. They allow the cells to use H_2 as an energy source and are called (H_2) uptake hydrogenases (generally termed Hup). The Hup hydrogenases and *Escherichia coli* hydrogenase-1 (Hya) are heterotrimeric enzymes consisting of a core heterodimer of an Fe – S cluster binding β-subunit (HupS, HyaA) and an α-subunit that binds the [NiFe] active site cofactor (HupL, HyaB). This associates with a third integral membrane cytochrome *b* γ-subunit (HupC, HyaC) to form the holoenzyme. The core hydrogenase dimer is anchored to the membrane by the di-heme cytochrome *b*

Fig. 2 Examples of electron transfer catalyzed by respiratory hydrogenases of group 1. Hypothetical mechanism of fumarate respiration with H_2 in *Wolinella succinogenes* (**a**), and in *Escherichia coli* (**b**), ~~in *Methanosarcina barkeri* (**c**)~~. **a** Electron and proton transfer in the membrane of *W. succinogenes* according to the "E pathway hypothesis" of Lancaster et al. (2005), which proposes that transmembrane electron transfer via the heme groups of the di-hemic quinol:fumarate reductase is strictly coupled to cotransfer of protons via a transiently established pathway, where the side chain of residue Glu-C180 plays a prominent role. The two protons that are liberated upon oxidation of menaquinol (MKH_2) are released to the periplasm. In compensation, coupled to electron transfer via the two heme groups, protons are transferred from the periplasm to the cytoplasm (via the ring C propionate of the distal heme and the residue Glu-C180 of the membrane subunit of fumarate reductase), where they replace those protons that are bound during fumarate reduction (Kröger et al. 2002; Lancaster et al. 2005). The HydC protein of the hydrogenase forms four transmembrane helices; the heme *b* groups are shown as *diamonds*. The menaquinone binding site is close to the distal heme *b* group, near the cytoplasmic side of the membrane (Gross et al. 2004). [4Fe – 4S] and [3Fe – S] clusters are represented by *squares* and the [2Fe – 2S] cluster by a *rectangle*. **b** In *E. coli*, hydrogenase-2 donates electrons to heme-less fumarate reductase. Unlike trimeric uptake hydrogenases with a membrane integral cytochrome *b* as third subunit, *E. coli* hydrogenase-2 is heterotetrameric; besides the $\alpha\beta$ heterodimeric core, it includes a "16Fe" ferredoxin (HybA), most closely related to the periplasmically oriented HmcB protein from *Desulfovibrio vulgaris* (Hildenborough) (Dolla et al. 2000), and a large integral membrane protein (HybB), most closely related to the HmcC protein from *D. vulgaris* and predicted to comprise ten transmembrane helices (Dubini 2002). **c** ~~the~~ The trimeric F_{420}-nonreducing hydrogenase (Vho) from *Methanosarcina mazei* Gö1, with a cytochrome *b* subunit that acts as the primary electron acceptor of the core hydrogenase, is shown to interact with the heterodisulfide reductase via methanophenazine (MP), the membrane integral electron carrier connecting protein complexes of the respiratory chain of *Ms. mazei*. The scheme shows that the membrane integral cytochrome *b* subunit accepts two protons from the cytoplasm for the reduction of MP and that the overall reaction leads to the production of two scalar protons (Ide et al. 1999), (adapted from Deppenmeier 2004)

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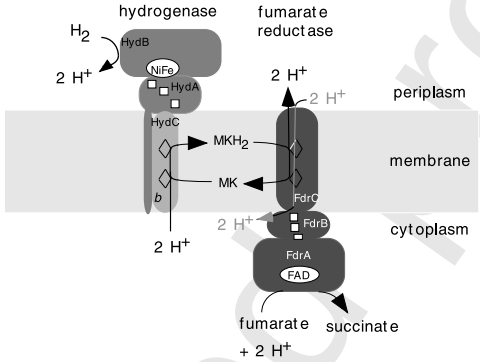
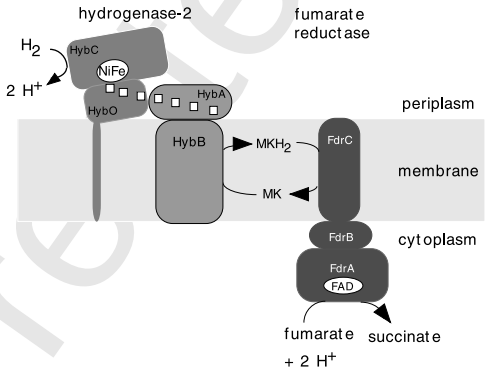
(Dross et al. 1992; Bernhard et al. 1997), which connects it to the quinone pool of the respiratory chain in the membrane, and by the hydrophobic C-terminus of the small subunit (Cauvin et al. 1991; Dross et al. 1992). The prototype, the hydrogenase of *Wolinella succinogenes*, encoded by the *hydABC* genes (thoroughly studied by the group of the late Achim Kröger) is shown in (Fig. 2a). Other members of group 1, such as the Hyn enzyme from *Thiocapsa roseop-*

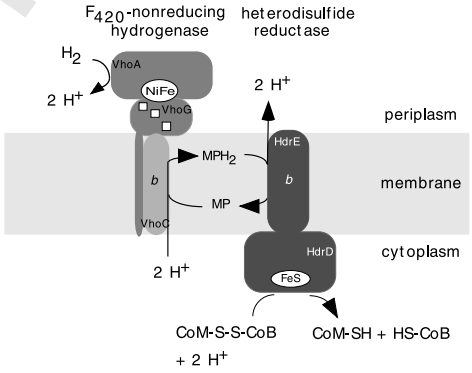
a) *W. Succinogenes*



b) *E. coli*



c) *Ms. barkeri*



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ersicina (Rákhely et al. 1998), the periplasmic *Desulfovibrio* hydrogenase able to interact with low-potential *c*-type cytochromes, and a transmembrane redox protein complex encoded by the *hmc* operon (Rossi et al. 1993) and *E. coli* hydrogenase-2 present a slightly different structure. *E. coli* hydrogenase-2 is predicted to be a large tetrameric complex consisting of the large (HybC) and the small (HybO) subunits associated to two other subunits, an Fe – S containing periplasmic subunit (HybA) and an integral membrane protein HybB (Dubini 2002) (Fig. 2b). Some *Desulfovibrio* species, e.g. *Desulfomicrobium baculatum* (formerly *Desulfovibrio baculatus*) contain a [NiFeSe]hydrogenase (HysSL). In this Se-containing hydrogenase, the carboxy-terminus of the gene encoding the large subunit contains a codon (TGA) for selenocysteine in a position homologous to a codon (TGC) for cysteine (Fauque et al. 1988). The SeCys in *Dm. baculatum* is a ligand to Ni (Garcin et al. 1999).

The uptake hydrogenases are characterized by the presence of a long signal peptide (30–50 amino acids residues) at the N-terminus of their small subunit. The signal peptide contains a conserved (S/T)RR~~z~~×F×K motif recognized by a specific protein translocation pathway known as membrane targeting and translocation (Mtt) (Weiner et al. 1998) or twin-arginine translocation (Tat) (Sargent et al. 1998; Rodrigue et al. 1999) pathway, and serves as signal recognition to target fully folded mature heterodimer to the membrane and the periplasm (Wu et al. 2000; Sargent et al. 2002). The twin-arginine motif has been shown to be required for successful assembly of the uptake hydrogenases from *Ralstonia eutropha* (Bernhard et al. 2000), and *W. succinogenes* (Gross et al. 1999). The Tat translocase transports fully folded proteins across the energy-transducing inner membrane using energy provided by the transmembrane Δp (Yahr and Wickner 2001). The Tat pathway is structurally and mechanistically similar to the ΔpH-dependent pathway used to import chloroplast proteins into the thylakoid (Mori and Cline 2001, 2002; Berks et al. 2003, 2005). Homologs of Tat proteins are found in many archaea, bacteria, chloroplasts, and mitochondria (Yen et al. 2002; ~~Palmer et al. 2005~~).

remove z to yield:
(S/T)RRxFxK

Palmer et al. 2005

2.1.2 Group 2

Group 2 hydrogenases are not exported and remain in the cytoplasm. In accordance, their small subunit does not contain a signal peptide at its N-terminus. They are subdivided into (i) the cyanobacterial uptake hydrogenases induced under N_2 fixing conditions (Appel and Schulz 1998; Tamagnini et al. 2002) and (ii) the regulatory hydrogenases, which function as H_2 sensors in the regulatory cascade that controls the biosynthesis of some eubacterial uptake hydrogenases (Friedrich et al. 2005; Vignais et al. 2005). The third hydrogenase of *Aquifex aeolicus*, a soluble enzyme that clusters with group 2a hydrogenases has been proposed to provide reductant to the reductive TCA cycle for CO_2 fixation (Brugna-Guiral et al. 2003).

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2.1.3 Group 3

In Group 3, the dimeric hydrogenase module is associated with other subunits able to bind soluble cofactors, such as cofactor 420 (F_{420}, 8-hydroxy-5-deazaflavin), NAD, or NADP. They are termed bidirectional hydrogenases for, physiologically, they function reversibly and can thus reoxidize the cofactors under anaerobic conditions by using the protons of water as electron acceptors. Many members of this group belong to the *Archaea* domain (Fig. 1).

Bidirectional NAD(P)-linked hydrogenases are also found in bacteria and in cyanobacteria. The first NAD-dependent [NiFe]hydrogenase was isolated from *R. eutropha* (formerly *Alcaligenes eutrophus* now renamed *Cupriavidus necator*) (Schneider and Schlegel 1976) in which it is encoded by genes located on a megaplasmid (Schwartz et al. 2003). Homologous NAD(P)-linked hydrogenases were later discovered in cyanobacteria and in the photosynthetic bacterium *T. roseopersicina* (Rákhely et al. 2004) (reviews by (Appel and Schulz 1998; Vignais et al. 2001; Tamagnini et al. 2002)). These bidirectional hydrogenases are composed of two moieties: the heterodimer [NiFe]hydrogenase encoded by the *hoxY* and *HoxH* genes and the diaphorase moiety, encoded by the *hoxU*, *hoxF* and *hoxE* genes, the products of which are homologous to subunits of complex I of the mitochondrial and bacterial respiratory chains and contain NAD(P), FMN, and Fe – S binding sites (Fig. 3, Table 1).

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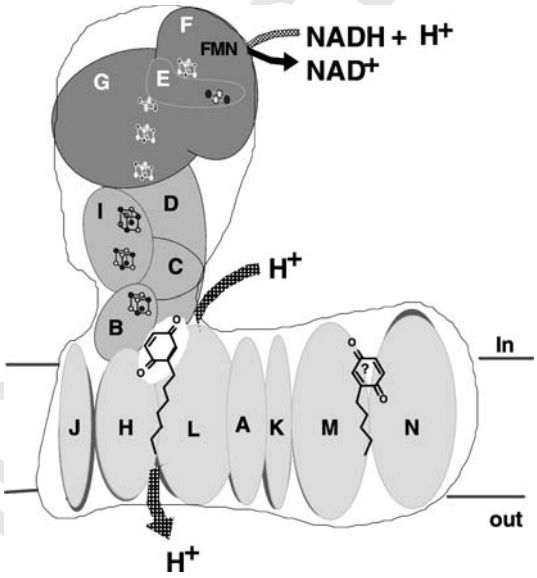

Fig. 3 Schematic representation of *Rhodobacter capsulatus* complex I. The [4Fe – 4S] and [2Fe – 2S] clusters are shown in the appropriate subunits (adapted from Dupuis et al. 2001; Holt et al. 2003; Sazanov and Hinchliffe 2006)

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F420 (subscript 420)
H2 (subscript 2)

Table 1 Relationships between complex I and NDH-1 subunits and subunits of selected [NiFe]hydrogenases and of F420H2 dehydrogenase

move HoxEFUYH to the right above H2ase

	Bovine[1]	*Synechocystis*[2]	*E. coli*[3] or *P. denitrificans*[5]	*E. coli*[6]	*M. barkeri*[7]	*R. rubrum*[8]	*P. furiosus*[9]	*Ms. Mazei*[10]
	Complex I	HoxEFUYH NDH-1 H2ase	NDH-1	NDH-1	Hyc H2ase	Ech H2ase	Coo H2ase	Mbh H2ase	Fpo
		subscript 2			subscript 2	subscript 2	subscript 2	subscript 2	
Hydrophilic NADH-oxidizing module	9 kDa								
	24 kDa	HoxE	NuoE	Nqo2					
	51 kDa	HoxF	NuoF	Nqo1					
	75 kDa	HoxU[a]	NuoG	Nqo3					
Subunits of the connecting module			NuoCD (*E.c.*)[b]		HycE				
	30 kDa	NdhJ	NuoC (*R.c.*)	Nqo5	N-ter HycE	EchD			FpoC
	49 kDa	NdhH HoxH	NuoD (*R.c.*)	Nqo4	C-ter HycE	EchE	CooH	Mbh12	FpoD
	20 kDa (PSST)	NdhK HoxY	NuoB	Nqo6	HycG	EchC	CooL	Mbh10	FpoB
	23 kDa (TYKY)	NdhI	NuoI	Nqo9	HycF	EchF	CooX	Mbh14	FpoI
	39 kDa								
	18 kDa								
	13 kDa B								
									FpoO
				(Nqo15[c])					
	~~39 kDa~~								

39 kDA should be moved to the next part of the Table

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move HoxEFUYH to the right above H2ase

add 39 kDa above ND1

Table 1 (continued)

		HoxEFUYH		*P. denitrificans*[5]	*E. coli*[6]	*M. barkeri*[7]	*R. rubrum*[8]	*P. furiosus*[9]	*Ms. Mazei*[10]
	Complex I	NDH-1 H2ase	NDH-1	NDH-1	Hyc H2ase	Ech H2ase	Coo H2ase	Mbh H2ase	Fpo
Intrinsic-membrane hydrophobic subunits	ND1	NdhA	NuoH	Nqo8	HycD	EchB	CooK	MbhL	FpoH
	ND2	NdhB	NuoN	Nqo14	HycC[d]	EchA[d]	N-ter CooM[d]	Mbh8	FpoN
	ND3	NdhC	NuoA	Nqo7					FpoA
	ND4	NdhD	NuoM	Nqo13	HycC[d]	EchA[d]	N-ter CooM[d]		FpoM
	ND4L	NdhE	NuoK	Nqo11					FpoK
	ND5	NdhF	NuoL	Nqo12	HycC[d]	EchA[d]	N-ter CooM[d]		FpoL
	ND6	NdhG	NuoJ	Nqo10					FpoJ

References:
[1] Fearnley and Walker 1992; [2] Kaneko et al. 1996; Schmitz et al. 2002; [3] Weidner et al. 1993; [4] Dupuis et al. 1998; [5] Yagi 1993; [6] Sauter et al. 1992; [7] Künkel et al. 1998; [8] Fox et al. 1996a,b; [9] *P. furiosus* genome database (http://comb5-156.umbi.umd.edu/) and Sapra et al. 2000; [10]Bäumer et al. 2000

[a] Sequence similarities between HoxU and N-ter NuoG

[b] NuoC and NuoD are fused in *E. coli* (Blattner et al. 1997)

[c] Nqo15 in *Thermus thermophilus* (Hinchliffe et al. 2006)

[d] NuoL, NuoM, and NuoN are homologous to one particular class of Na^+/H^+ antiporters (Hamamoto et al. 1994)

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2.1.4 Group 4

In Group 4 cluster multimeric (six subunits or more) membrane-bound hydrogenases, which comprise transmembrane subunits homologous to complex I subunits involved in proton pumping and energy coupling (Table 1, Fig. 4). They appear to be able to couple the oxidation of a carbonyl group (originating from formate, acetate, or carbon monoxide) with the reduction of protons to H_2 and form the group of *energy-converting, H_2-evolving hydrogenases*. The prototype of this group is *E. coli* hydrogenase-3, encoded by the *hyc* operon, part of the formate hydrogen lysase complex (FLH-1) (Böhm et al. 1990; Sauter et al. 1992), which metabolizes formate to H_2 and CO_2, the biosynthetic pathway of which has been deciphered by the group of A. Böck (Sawers et al. 2004). *E. coli* also contains the *hyf* operon, which can encode a putative 10-subunit hydrogenase complex (hydrogenase-4); seven of the *hyf* genes encode homologs of seven Hyc subunits of hydrogenase-3. Three additional genes (*hyfD, hyfE* and *hyfF*) have no counterpart in the Hyc complex

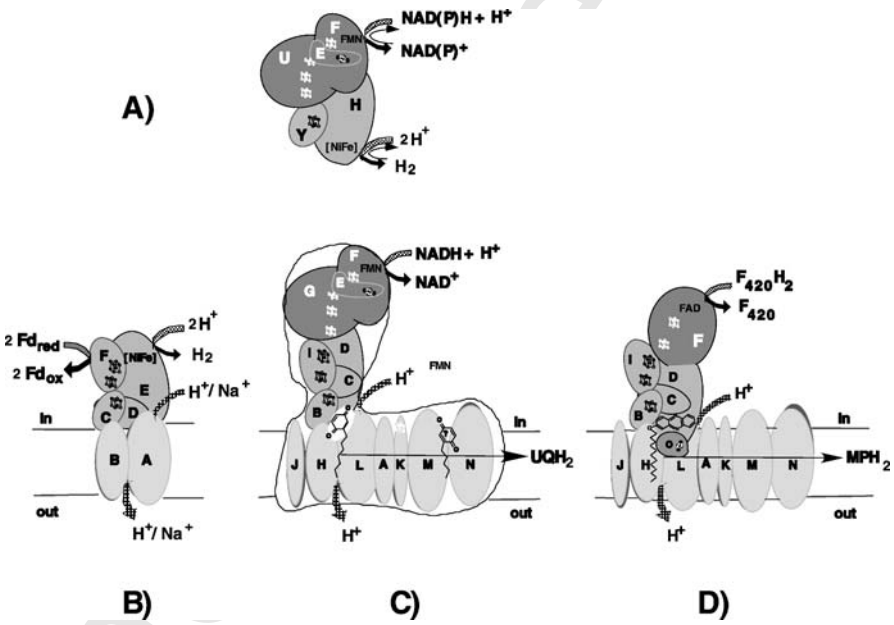

Fig. 4 Models of [NiFe]hydrogenases and $F_{420}H_2$ dehydrogenase compared with that of complex I from *Rhodobacter capsulatus* (**c**). **a** Bidirectional Hox hydrogenase from *Synechocystis* encoded by the *HOXFUYH* genes. **b** Hydrogenase from *Methanosarcina barkeri*, encoded by the *echABCDEF* genes (adapted from Hedderich 2004). **d** $F_{420}H_2$ dehydrogenase from *Methanosarcina mazei* encoded by the *fpoA-O* genes (adapted from Deppenmeier 2004)

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and are capable of encoding integral membrane proteins, two of them sharing similarities with subunits that play a crucial role in proton translocation and energy coupling in the NADH:quinone oxidoreductase (complex I) (Andrews et al. 1997). Up to now, no Hyf-derived hydrogenase activity could be detected and no Ni-containing protein corresponding to HyfG, the large subunit of hydrogenase-4 has been observed (Skibinski et al. 2002).

The CO-induced hydrogenase of the photosynthetic bacterium *Rhodospirillum rubrum* is another member of group 4. It is part of the CO-oxidizing system that allows *R. rubrum* to grow in the dark with CO as sole energy source. CO dehydrogenase and the hydrogenase encoded by the *coo* operon oxidize CO to CO_2 with concomitant production of H_2. Since the CO dehydrogenase is a peripheral membrane protein, it was proposed that the hydrogenase component of the oxidizing system constitutes the energy coupling site (Fox et al. 1996). A homologous CO-oxidizing complex has been isolated from the thermophilic Gram-positive bacterium *Carboxydothermus hydrogenoformans* (Soboh et al. 2002).

Group 4 hydrogenases were later isolated from *Archaea* and shown to be able to couple H_2 evolution and energy conservation. They include the EhA and Ehb hydrogenases from *Methanothermobacter* species (Tersteegen and Hedderich 1999), the Ech hydrogenase from *Methanosarcina barkeri* (Künkel et al. 1998; Meuer et al. 1999), and the Mbh hydrogenase from *Pyrococcus furiosus* (Sapra et al. 2000; Silva et al. 2000; recent reviews by Hedderich 2004; Hedderich and Forzi 2005; Vignais and Colbeau 2004). Some, found in present-day hyperthermophiles, were acquired from *Archaea* by horizontal gene transfer. According to Calteau et al. (2005) this would be the case for the 13-gene operon found in the genome of *Thermotoga maritima*, capable of encoding a Mbx hydrogenase, probably acquired by horizontal transfer from an archaebacterium belonging to the *Pyrococcus* group, and for the six-gene *ech* operon found in *Thermoanaerobacter tengcongensis* (Sobboh et al. 2004) and in *Desulfovibrio gigas* (Rodrigues et al. 2003), which was probably transferred independently from an archaebacterium belonging to the *Methanosarcina* clade.

2.2 The [FeFe]hydrogenases

[FeFe]hydrogenases are found in anaerobic prokaryotes, such as clostridia and sulfate reducers, and in eukaryotes (see reviews by Adams 1990; Atta and Meyer 2000; Vignais et al. 2001; Horner et al. 2000, 2002). [FeFe]hydrogenases are the only type of hydrogenases to have been found in eukaryotes, and they are located exclusively in membrane-limited organelles, i.e., in chloroplasts or in hydrogenosomes. They are usually involved in H_2 production.

Unlike [NiFe]hydrogenases composed of at least two subunits, many [FeFe]hydrogenases are monomeric and consist of the catalytic subunit only,

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although dimeric, trimeric, and tetrameric enzymes are also known (Vignais et al. 2001). The catalytic subunit of [FeFe]hydrogenases, in contrast to those of Ni-containing enzymes, vary considerably in size. Besides the conserved domains of ca. 350 residues containing the active site (H-cluster, Adams 1990), they often comprise additional domains, which accommodate Fe – S clusters (Fig. 5). The H-cluster consists of a binuclear iron subsite ($[Fe_2S_3]$) bound to a conventional [4Fe – 4S] cluster by a bridging cysteinyl sulfur. To each Fe atom a terminal carbon monoxide, a bridging carbon monoxide, and a cyanide ligand are bound. The Fe atoms also share two bridging sulfur ligands of a di(thiomethyl)amine molecule $(CH_3 - S^-)_2$ (Peters et al. 1998, 1999; Nicolet et al. 1999, 2000, 2002).

Although [FeFe]- and [NiFe]hydrogenases have completely different structures and are evolutionary unrelated, they share a common feature, namely the presence of endogenous CO and CN^- ligands bound to a Fe center in the active site. The presence of these ligands stabilizes iron in a low oxidation and

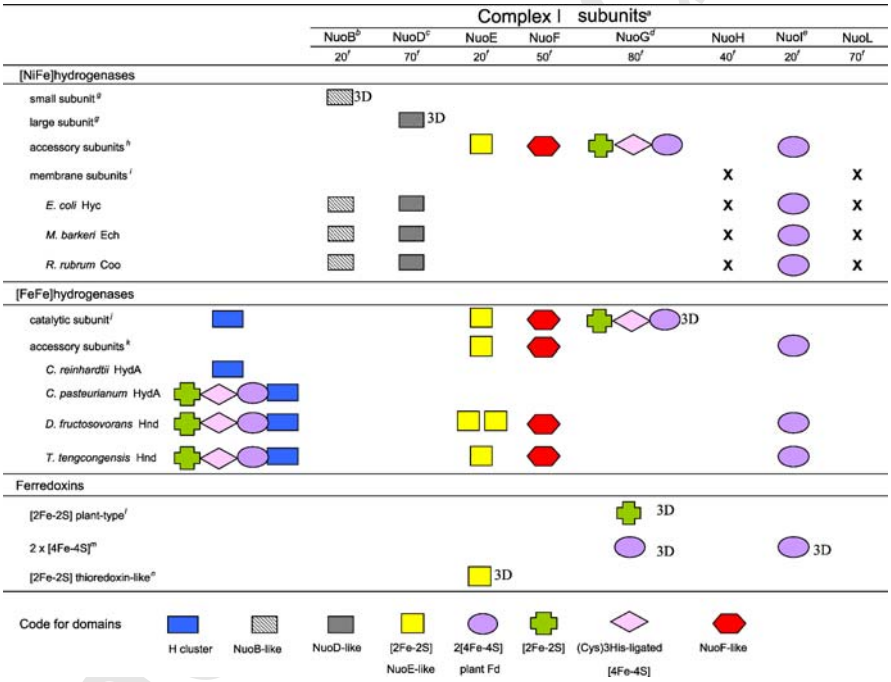

Fig. 5 Schematic representation of homologies between hydrogenases and complex I. The code for domains is indicated in the *lower part* of the figure. [a] Complex I subunits are designated by the *nuo* nomenclature used for *E. coli* and *Rb. capsulatus*. [b] Approximate masses (kDa) of subunits found in various bacteria. The H-cluster domain is included although it has no homolog in complex I (adapted from Fig. 10 of Vignais et al. (2001), where a detailed legend can be consulted)

only accessory subunits of some [FeFe]hydrogenases have

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spin state and makes it resemble transition metals (Ru, Pd, or Pt) known to be good catalysts for H_2 splitting (Adams and Stiefel 2000). Another common feature is the presence of an Fe – S cluster proximal to the dinuclear metallocenter, which is then wired to the surface for electron exchange with its partner redox proteins by a conduit of Fe – S clusters. Finally, both types of enzymes contain hydrophobic gas channel(s) that runs from the molecular surface to the buried active site (Nicolet et al. 2002).

A [FeFe]hydrogenase is proposed to have been a key enzyme at the origin of the eukaryotic cell. Two hypotheses posit that a metabolic symbiosis (syntrophy) between a methanogenic archaebacterium and a proteobacterium able to release H_2 in anaerobiosis was the first step in eukaryogenesis. The hydrogen hypothesis (Martin and Müller 1998) proposes that an anaerobic heterotrophic *α-Proteobacterium*, producing H_2 and CO_2 as waste products, formed a symbiotic metabolic association (syntrophy) with a strictly anaerobic, autotrophic archaebacterium, possibly a methanogen dependent on H_2. The intimate relationship over long periods of time allowed the symbiont and the host to co-evolve and become dependent on each other. In an anaerobic environment the symbiont was either lost, as in type I amitochondriate eukaryotes, or became a hydrogenosome (i.e., a hydrogen-generating and ATP-supplying organelle) as in type II amitochondriate eukaryotes (Müller 1993). By further evolution, the host lost its autotrophic pathway and its dependence on H_2 and the endosymbiont adopted a more efficient aerobic respiration to become the ancestral mitochondrion. Thus, the eukaryotic cell would have emerged as the result of endosymbiosis between two prokaryotes, an H_2-dependent, autotrophic archaebacterium (the host) and an H_2- and ATP-producing eubacterium (the symbiont), the common ancestor of mitochrondria and hydrogenosomes. The syntrophy hypothesis for the origin of eukaryotes, proposed at the same time and independently (Moreira and López-Garcia 1998; López-Garcia and Moreira 1999) is based on similar metabolic consideration (interspecies hydrogen transfer), but the latter authors speculated that the organisms involved in syntrophy with methanogenic archaea were *δ-Proteobacteria* (ancestral sulfate-reducing myxobacteria) (it was also suggested that a second anaerobic symbiont was involved in the origin of mitochondria). Thus, hydrogenosomes are either considered to be relics of ancestral endosymbiont and to share a common origin with mitochondria (Bui et al. 1996; Martin and Müller 1998) or to have evolved several times as adaptations of mitochondria to anaerobic environments (Hackstein 2005; Hackstein et al. 2001; Embley et al. 2003; Tjaden et al. 2004).

Eukaryotic organelles contain only [FeFe]hydrogenases. A phylogenetic analysis of eukaryotic [FeFe]hydrogenases (Horner et al. 2000, 2002) suggests a polyphyletic origin of these enzymes, implying an acquisition by lateral gene transfer from different prokaryotic sources. On the other hand, the [FeFe]hydrogenases from green algae emerge as a monophyletic group

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with hydrogenosomal [FeFe]hydrogenases from microaerophilic protists (Horner et al. 2002). The source of an ancestral [FeFe]hydrogenase is not resolved; its presence in eukaryotes may reflect an early lateral transfer from a eubacterium. The plastidial [FeFe]hydrogenases appear to have a non-cyanobacterial origin, since cyanobacteria, the progenitors of chloroplasts, contain only [NiFe]hydrogenases and no [FeFe]hydrogenases (Vignais et al. 2001; Tamagnini et al. 2002). Eukaryotes possess genes that encode proteins that are phylogenetically related to [FeFe]hydrogenases. Mitochondria do not contain [FeFe]hydrogenase but have kept a key enzyme, cysteine desulfurase (called IscS or Nfs1), which performs a crucial role in cellular Fe – S protein maturation (Mühlenhoff and Lill 2000; Lill and Mühlenhoff 2005) and appears to have originated from the ancestor endosymbiont.

2.3 Hydrogenases and Complex I

The energy-converting NADH-ubiquinone oxidoreductase is the main entry site of reducing equivalents into the mitochondrial and the bacterial respiratory chains (for a recent review see Brandt 2006). The mitochondrial enzyme is also called complex I, whereas the bacterial enzyme is more often referred to as type 1 NADH-dehydrogenase or NDH-1. The bovine mitochondrial complex I contains a total of 46 different subunits (Carroll et al. 2003; Hirst et al. 2003) whereas NDH-1 from the bacteria *Paracoccus denitrificans* (Yagi 1993; Yagi et al. 1998) and *Rhodobacter capsulatus* (Dupuis et al. 1998) contain 14 subunits, all of which have homologs in the bovine enzyme (Table 1). Both the mitochondrial and bacterial enzymes are L-shaped, with a membrane domain and a peripheral arm extending into the cytosol. The hydrophilic NADH-oxidizing module, distal from the membrane comprises three hydrophilic subunits containing FMN and five Fe – S clusters; a second hydrophilic module consisting of four subunits connects the NADH-oxidizing proteins to the membrane-bound hydrophobic subunits. The two extramembranous modules contain all the redox centers of the enzyme (Dupuis et al. 1998, 2001; Yagi et al. 1998; Friedrich 2001; Friedrich et al. 1998; Schultz and Chan 2001; Sazanov et al. 2000; Sazanov and Hinchliffe 2006) (Fig. 3). Sequence similarities between hydrogenases and complex I, first reported by Böhm et al. (1990) and Pilkington et al. (1991), have been emphasized in several subsequent reports (Friedrich and Weiss 1997; Friedrich and Scheide 2000; Albracht and Hedderich 2000; Dupuis et al. 2001; Friedrich 2001; Yano and Ohnishi 2001; Vignais et al. 2001, 2004; Hedderich 2004). Subunits NuoE, NuoF, NuoI, and the N-terminal Fe – S binding domain (ca. 220 residues) of NuoG have homologous counterparts in accessory subunits and domains of soluble [NiFe]hydrogenases of group 3 (Hox) and group 4 (Ech) and of [FeFe]hydrogenases (Fig. 5). In addition, three subunits located within Fig. 4,

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the connecting module of complex I share similarities with subunits of the core [NiFe] enzyme, the NuoB subunit with the small hydrogenase subunit (the [4Fe – 4S] cluster of NuoB, known as cluster N2 (Ohnishi et al.1998) and suggested to be a key component in redox-driven proton translocation (Flemming et al. 2005) is related to the hydrogenase proximal cluster), and the NuoC and NuoD subunits (fused as a single NuoCD protein in *E. coli*) with the large subunit. Furthermore, hydrophobic subunits of multimeric, membrane-bound [NiFe]hydrogenases belonging to group 4, e.g., *E. coli* Hyc and Hyf, *R. rubrum* Coo, *Ms. barkeri* Ech, *Methanothermobacter marburgensis* Eha and Ehb and *P. furiosus* Mbh are also homologous to transmembrane subunits of complex I (NuoH, NuoL, NuoM, NuoN). It should be noted that these hydrogenases of group 4 are also ion (H^+ or Na^+) pumps (the nature of the coupling ion used is still elusive). Thus, the presumed evolutionary links between hydrogenases and complex I concern not only the electron transferring subunits but also the ion pumping units, i.e., the coupling between electron transport and energy recovery by a chemiosmotic mechanism.

On the basis of the similarities between [NiFe]hydrogenases and the NuoB–NuoD dimer of the connecting module, Dupuis et al. (2001) have proposed (i)that the [NiFe] active site of hydrogenases was reorganized into a quinone-reduction site carried by the NuoB–NuoD dimer (Prieur et al. 2001) and a hydrophobic subunit such as NuoH (Fig. 3), and (ii) that NuoD might provide both the quinone gate and a potential proton channel entry for a minimal "proton pumping" module composed of subunits NuoB, NuoD, NuoI, and NuoL (Friedrich and Scheide 2000; Dupuis et al. 2001; see also Kashani-Poor et al. 2001). Subunit NuoL (or NuoN or NuoM, considered up to recently to have evolved by triplication of an ancestral gene related to bacterial Na^+ or K^+/H^+ antiporters (Fearnley and Walker 1992; Friedrich and Weiss 1997)) would have provided the transmembrane channel required to complete the proton (or Na^+) pump (Dupuis et al. 2001). These membrane proteins are similar to an electrogenic Na^+/H^+ antiporter first identified in an alkalophilic *Bacillus* strain (Hamamoto et al. 1994). In *Bacillus subtilis* the corresponding proteins are encoded by a seven-gene operon, *mrp* (multiple resistance and pH), and are termed MrpA-G (Ito et al. 1999) (the *sha* nomenclature for "sodium hydrogen antiporter" is also used (Kosono et al. 2006)). The MrpA and the MrpD antiporters come in two subclasses, MrpA-type and MrpD-type, and it has been determined that NuoL is more closely related to MrpA and that NuoM and N are more closely related to the MrpD antiporter (Mathiesen and Hägerhäll 2002). NuoK has later been shown to be homologous to MrpC, suggesting that a multisubunit antiporter complex was recruited to the ancestral enzyme (Mathiesen and Hägerhäll 2003). The latter authors concluded that the last common ancestor of complex I and the membrane-bound [NiFe]hydrogenases of group 4 contained the NuoKLMN subunit module.

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The nature of the ion translocated by complex I (Na^+ or H^+) is still a matter of debate. The mitochondrial enzyme of respiratory chains were shown to be proton pumps (Hinkle 2005) but some bacterial respiratory enzymes generate an Na^+ gradient (Dimroth and Cook 2004) and many marine and pathogenic bacteria have a sodium-translocating NADH:ubiquinone oxidoreductase, which generates an electrochemical Na^+ gradient during aerobic respiration (Barquera et al. 2004). Complex I from *Klebsiella pneumoniae* (Krebs et al. 1999) and from *E. coli* (Steuber 2001) have been proposed to work as an Na^+ pump. Since the membranous complex I subunits NuoL, NuoM, and NuoN are homologous to cation/proton antiporters (Friedrich and Scheide 2000; Mathiesen and Hägerhäll 2002, 2003) the question arises whether the complex is involved in primary proton translocation or is capable of secondary Na^+/H^+ antiport. Recently, Stolpe and Friedrich (2004) have shown that *E. coli* complex I is a primary proton pump but is capable of secondary sodium antiport.

3
Modes of Energy Conservation by Hydrogenases

3.1
Energy Conservation via Energy-Transducing Electron Transport Chains by Respiratory [NiFe]hydrogenases (Group 1)

The uptake hydrogenases link the oxidation of H_2 to the reduction of oxygen (aerobic respiration) or to the reduction of anaerobic electron acceptors such as NO_3^-, SO_4^{2-}, fumarate, and TMAO/DMSO (anaerobic respiration). Similarly to other substrate-specific dehydrogenases they feed electrons into a common quinone pool, from which electrons are transferred via specific quinol oxidases to terminal reductases, e.g., in the absence of oxygen, TMAO/DMSO, nitrate or fumarate reductase and, in the presence of oxygen, cytochrome oxidase. The oversimplified scheme of Fig. 6 is meant to emphasize the role of the quinone pool in respiration, the type of quinone (e.g., ubiquinone, UQ, menaquinone, MK) depending on the prevailing environmental conditions (Richardson 2000). The third subunit of the trimeric uptake hydrogenase, the di-heme cytochrome *b* encoded by *hupC* in *Rb. capsulatus* and *hoxZ* in *R. eutropha* and *Azotobacter vinelandii* is the necessary link for transfer of electrons from H_2 to the electron transport chain; furthermore, it plays a role in activating and maintaining the hydrogenase in a reduced, active state (Cauvin et al. 1991; Sayavedra-Soto and Arp 1992; Bernhard et al. 1997; Meek and Arp 2000). Electrons from H_2 are donated to the quinone pool (Henry and Vignais 1983) and the energy of H_2 oxidation is recovered by vectorial proton transfer at the level of the quinol oxidase (Kömen et al. 1996), cytochrome bc_1 complex and cytochrome ox-

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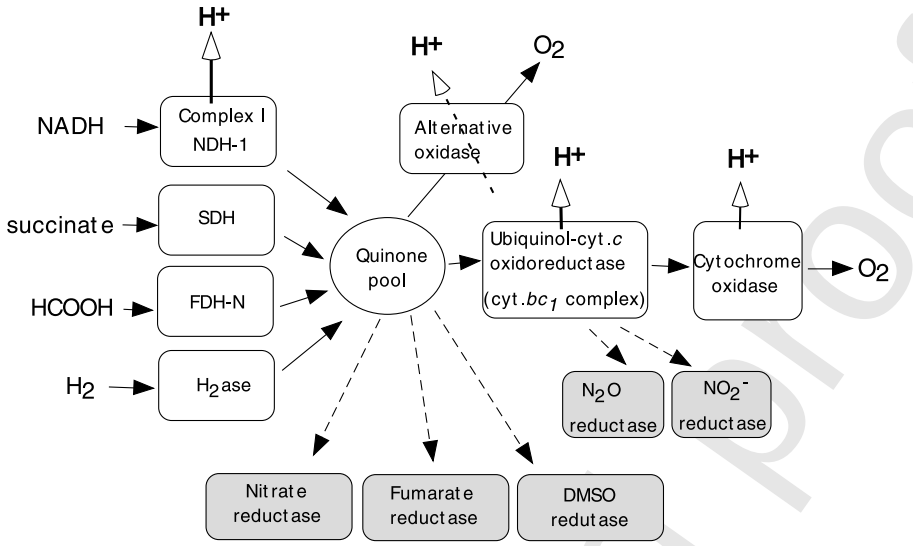

Fig. 6 Simplified and general scheme illustrating electron pathways in respiratory chains. *White boxes* indicate electron-input units and *black arrows* the influx of reducing equivalents in the membrane. The respiratory hydrogenase represented here is usually trimeric, the third subunit being a di-heme cytochrome *b*, which anchors the hydrogenase to the membrane, binds the quinone, and is the link for the transfer of H_2 electrons to the quinone (Cauvin et al. 1991; Bernhard et al. 1997; Gross et al. 2004). *Dashed arrows* indicate electron flux to output modules involved in anaerobic respiration (*shaded boxes*). Energy coupling sites are indicated by *arrows* showing vectorial proton ejection. Not shown is the Δp created across the membrane when fumarate reductase is reduced via the quinone (menaquinone, MK) with H_2 or formate (Kröger et al. 2002). The proton pumping activity of alternative oxidase (cyt *bo*-type) (Kömen et al. 1996) is indicated by a *dashed arrow* since it is not a common case among the quinol oxidases

idase (Paul et al. 1979; Porte and Vignais 1980). *W. succinogenes* performs oxidative phosphorylation with fumarate as terminal electron acceptor and H_2 (or formate) as electron donor. This fumarate respiration, catalyzed by an electron transport chain consisting of hydrogenase, menaquinone, and fumarate reductase (Fig. 2a), is coupled to the generation of an electrochemical proton potential (Δp) across the bacterial membrane generated by MK reduction with H_2 (Kröger et al. 2002; Lancaster et al. 2005). In the methanogenic archaeon *Methanosarcina mazei* Gö1, the VhoGA uptake hydrogenase transfers electrons from H_2 to a cytochrome *b* (VhoC); the electrons are then channeled through methanophenazine to heterodisulfide reductase, which reduces the CoM–S–S–CoB heterodisulfide to produce CoB–SH, the reductant for the formation of methane from methyl–S–CoM (Ide et al. 1999) (Fig. 2c, Fig. 7).

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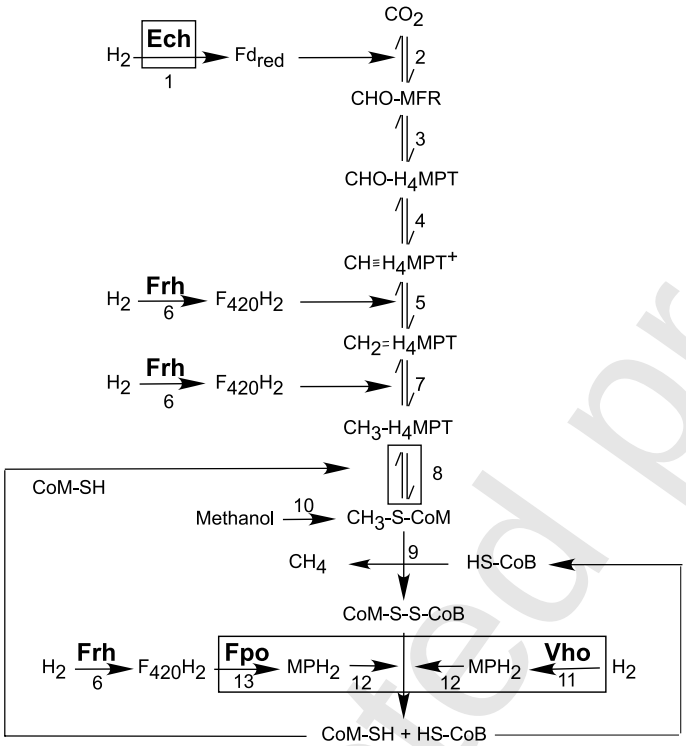

Fig. 7 Pathway of methanogenesis from $CO_2 + H_2$ and from methanol. Reactions catalyzed by membrane-bound energy-transducing enzyme complexes are *boxed*.
Abbreviations: $F_{420}H_2$ reduced form of coenzyme F_{420}; Fd_{red} reduced form of ferredoxin; MFR methanofuran; H_4MPT tetrahydromethanopterin ; HS-CoM coenzyme M; HS-CoB coenzyme B; MPH_2 reduced form of methanophenazine.
Enzymes: *1* Ech, Ech hydrogenase; *2* formylmethanofuran dehydrogenase; *3* formyl-MFR:H_4MPT formyl transferase; *4* methenyl-H_4MPT cyclohydrolase; *5* methylene-H_4MPT dehydrogenase; *6* Frh, F_{420}-reducing hydrogenase; *7* methylene-H_4MPT reductase; *8* methyl-H_4MPT:HS-CoM methyltransferase; *9* methyl-CoM reductase; *10* soluble methyltransferases; *11* Vho, F_{420}-nonreducing hydrogenase; *12* heterodisulfied reductase; *13* Fpo, $F_{420}H_2$ dehydrogenase. Each type of hydrogenase with its specific electron acceptor (ferredoxin for Ech, cofactor F_{420} for Frh, and me highlighted in bold is shown ~~*highlighted in bold*~~ (adapted from Deppenmeier 2004)

heterodisulfide

3.2 Energy-Conservation by Proton/Na^+ Translocation by Membrane-Bound, H_2 Evolving [NiFe]hydrogenases (Group 4)

The mechanism of energy conservation by Na^+/H^+ translocation has been best studied with [NiFe]hydrogenases from methanogens, which obtain most or all their energy for growth from the process of methane biosynthesis

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(methanogenesis), considered to be an anaerobic respiration (see ~~Chapter 6~~ in this volume[CE: b] and reviews by Deppenmeier 2002, 2004; Hedderich 2004; Hedderich and Forzi 2005). V. Müller

Strictly anaerobic archaea of the genus *Methanosarcina* derive their metabolic energy from the conversion to methane of a restricted number of C_1 compounds and acetate (Deppenmeier 2002). Figure 7 shows how CH_4 is formed from $CO_2 + H_2$ via the CO_2-reducing pathway, or from methanol. Three types of [NiFe]hydrogenases, identified recently (see reviews by Hedderich 2004; Deppenmeier 2004), are involved in these two systems in which either H_2 or $F_{420}H_2$ are used as electron donor and the heterodisulfide CoM–S–S–CoB as electron acceptor (hence the term "disulfide respiration" used by Hedderich and Whitman (2005)). H_2 reduction of low-potential ferredoxin by Ech, thermodynamically unfavorable, requires the consumption of a membrane ion gradient and thus occurs by so-called reverse electron transport. Redox-driven proton translocation catalyzed by intrinsic membrane subunits of the Ech hydrogenase and Fpo dehydrogenase generates a proton-motive force and hence energy recovery during methanogenesis (Fig. 7). In acetoclastic methanogenesis, Ech couples the oxidation of reduced ferredoxin (arising from the oxidation of the carbonyl group of acetate) to the production of H_2.

Methanophenazine (MP), which acts in the membrane of the methanogen as the quinone in respiratory chains of bacteria and mitochondria, can be reduced either with H_2, by the F_{420}-non reducing hydrogenase VhoAG via its third subunit, VhoC, which interacts with MP (Fig. 2c), or with $F_{420}H_2$ by the $F_{420}H_2$ dehydrogenase (FpoDH), a multimeric complex encoded by the *fpo* genes, with subunits homologous to subunits of complex I (Table 1) (Fig. 4). The heterodisulfide reductase (HdrED) receives electrons from the reduced form of methanophenazine, MPH_2 (Fig. 2c). Each partial reaction, the reduction of MP by H_2 or $F_{420}H_2$ and the reduction of CoM–S–S–CoB by MPH_2 is coupled to the translocation of $2H^+/2e^-$. H^+-translocation in both reactions can occur via a redox-loop mechanism, while $F_{420}H_2$dehydrogenase is thought to function as a proton pump (Ide et al. 1999; Bäumer et al. 2000).

Another member of group 4, the Mbh hydrogenase from *P. furiosus*, has been shown to couple electron transfer from reduced ferredoxin to both proton reduction and proton translocation. Oxidation of reduced ferredoxin by inverted membrane vesicles of *P. furiosus* generated both a $\Delta\psi$ and a ΔpH, which could be coupled to ATP synthesis (Sapra et al. 2003)

3.3 Disposal of Excess Reducing Equivalents

Growth of bacteria depends on dissimilatory and assimilatory processes. Oxidation of inorganic or organic substrates results in the formation of reducing power (NADH) and ATP, which is used to drive assimilatory processes leading

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to the synthesis of cell materials. Growth rates depend on ATP content and the (photo)phosphorylation rate is regulated by redox balance. "Over-reduction" or "over-oxidation" of the redox components of the electron transport chain (including the quinone pool) leads to inhibition of phosphorylation (Bose and Gest 1963). The requirement for a membrane redox poise close to the oxidation–reduction potential of the ubiquinone pool (Candela et al. 2001) can be explained by the involvement of a semiquinone intermediate in the Q cycle (Nicholls and Ferguson 1992; Dutton et al. 1998; Brandt 1999). To dissipate excess reducing equivalents from the photosynthetic membrane, bacteria such as *Rb. capsulatus* can use an alternative quinol oxidase, which allows the cell to control the redox state of the Q pool and the rate of photophosphorylation activity (Zannoni and Marrs 1981). In *Rb. capsulatus*, under anaerobic conditions in the light, excess reducing equivalents are transferred to NAD^+ by reverse electron flow through complex I (Klemme 1969; Dupuis et al. 1997). Reducing equivalents stored in NADH can be dissipated by metabolic systems such as CO_2 fixation (Calvin cycle), nitrogen fixation, or reduction of auxiliary oxidants (Hillmer and Gest 1977; Tichi et al. 2001). In the case of nitrogen fixation, which is catalyzed by nitrogenase, H_2 is produced as an intrinsic part of the enzymatic reaction and, in the absence of N_2, nitrogenase functions as a hydrogenase, reducing protons to H_2 (Vignais et al.1985; Willison 1993). Since nitrogenase is an ATP-dependent enzyme, this reaction dissipates energy as well as offering another means for disposal of excess reducing equivalents.

[NiFe]hydrogenases of group 3, which bind reduced coenzymes such as NADH, NADPH, and $F_{420}H_2$, can directly regenerate the oxidized coenzymes by using the protons of water as electron acceptors and then evolving H_2. H_2 production catalyzed by the cytoplasmic NAD(P)-dependent bidirectional [NiFe]hydrogenase (Hox) has indeed been observed with the cyanobacterium *Synechocystis* PCC6803. Significant H_2 production was observed when cells achieved anaerobiosis, the rate of H_2 production being higher in the presence of fermentative substrates such as glucose. The transient H_2 burst observed upon re-illumination probably reflected the increase in NAD(P)H concentration in response to photosystem I activity (Cournac et al. 2004). Appel et al. (2000) have proposed that the bidirectional hydrogenase functions as an electron valve for the disposal of low-potential electrons generated at the onset of illumination. Similarly, H_2 production has been observed when dark-adapted *Chlamydomonas reinhardtii* cells are illuminated (Cournac 2002). In that case, it is a [FeFe]hydrogenase which transfers electrons from a [2Fe – 2S] ferredoxin reduced by photosystem I. The [FeFe]hydrogenase is an electron "valve" that enables the algae to survive under anaerobic conditions (Happe et al. 2002).

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4 Conclusions and Perspectives

Hydrogenases are a structurally and functionally diverse group of enzymes, and phylogenetic analysis has led to the identification of several phylogenetically distinct groups and subgroups that form the basis of a coherent system of classification. Their modular structure, their additional domains and subunits that have counterparts in other redox proteins and complexes, has long been a matter of speculation. Their relationships with NADH-ubiquinone oxidoreductase (complex I) of respiratory chains has gained renewed interest with the recently identified multisubunit, membrane-bound, energy-conserving [NiFe]hydrogenases of methanogenic archaea.

Whole genome sequencing is not only increasing significantly the number of available hydrogenase sequences but is also revealing the presence of multiple hydrogenases in *Bacteria* and *Archaea*. Postgenomic analysis (transcriptome, proteome, metabolome) has and will be essential for elucidating the metabolic roles of these enzymes and the regulation of their biosynthesis and activity. The chief role of [NiFe]hydrogenases is clearly the oxidation of H_2 or the reduction of protons, coupled to energy-conserving electron transfer chain reactions, which allow energy to be obtained either from H_2 or from the oxidation of substrates of lower potential. In the last decade, additional roles have been revealed. Thus, the so-called H_2 sensors hydrogenases are involved in regulating the biosynthesis of uptake [NiFe]hydrogenases in response to their substrate, H_2. Other, bidirectional hydrogenases able to bind directly reduced coenzymes and re-oxidize them using protons from water as electron acceptors can act as electron "valves" to control the redox poise of the respiratory chain at the level of the quinone pool. This is essential to ensure the correct functioning of the respiratory chain in the presence of excess reducing equivalents, in particular in photosynthetic microorganisms. Finally, hydrogenases from group 4, those originally thought to play a purely fermentative role and the newly discovered ones in methanoarchaea, are now known to be involved in membrane-linked energy conservation through the generation of a transmembrane proton-motive force. protonmotive

The broad distribution of hydrogenases among existing microorganisms attests to the importance of H_2 metabolism in a wide range of environments, and suggests that hydrogenases may have appeared very early in evolution. Two newly formulated hypotheses propose that H_2 metabolism may have been the driving force that led to cellular symbiosis and fusion events involved in the formation of the first eukaryotic cells. The present day [FeFe]hydrogenases that are found in the organelles of unicellular eukaryotes (hydrogenosomes, chloroplasts) may be relics of these evolutionary events or the results of more recent lateral gene transfers. Their evolutionary origins is still unresolved and are the subject of current studies and debates. The discovery of hydrogenase-like sequences in the genomes of aerobic eukaryotes,

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including mammals, opens a new field of research. The encoded proteins, related to [FeFe]hydrogenases, appear to be involved in the maturation of Fe – S clusters, for insertion into the Fe – S proteins that are crucial for all cellular life. The cysteine desulfurase, a key enzyme of this pathway, located in the mitochondrion, appears to have originated from the mitochondrial endosymbiont. Comparative biochemical and genetic studies and determination of the localization of these proteins in hydrogenosomes and mitochondria will help to find out the reason why the host cell kept the endosymbiont: was it because of its ability to make Fe – S clusters for its host?

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