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MINIREVIEW

Atmospheric Hydrogen Scavenging: from Enzymes to Ecosystems

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We have known for 40 years that soils can consume the trace amounts of molecular hydrogen (H_2) found in the Earth's atmosphere. This process is predicted to be the most significant term in the global hydrogen cycle. However, the organisms and enzymes responsible for this process were only recently identified. Pure culture experiments demonstrated that several species of *Actinobacteria*, including streptomycetes and mycobacteria, can couple the oxidation of atmospheric H_2 to the reduction of ambient O_2. A combination of genetic, biochemical, and phenotypic studies suggest that these organisms primarily use this fuel source to sustain electron input into the respiratory chain during energy starvation. This process is mediated by a specialized enzyme, the group 5 [NiFe]-hydrogenase, which is unusual for its high affinity, oxygen insensitivity, and thermostability. Atmospheric hydrogen scavenging is a particularly dependable mode of energy generation, given both the ubiquity of the substrate and the stress tolerance of its catalyst. This minireview summarizes the recent progress in understanding how and why certain organisms scavenge atmospheric H_2. In addition, it provides insight into the wider significance of hydrogen scavenging in global H_2 cycling and soil microbial ecology.

It is well established that soil ecosystems can consume the trace concentrations of molecular hydrogen (H_2) found in the lower atmosphere (troposphere) (1, 2). As both the most significant and least understood process in the global biogeochemical cycle of H_2, this process has inspired interest among atmospheric chemists for four decades (3). The H_2 cycle is also relevant for climate change research, since the partial pressure of tropospheric H_2 affects the oxidative capacity of the atmosphere and the amount of water vapor in the stratosphere (3). However, only in the last few years have scientists started to look beyond the geochemical aspect and focus on the biological role of atmospheric H_2 scavenging. It is now clear that certain soil bacteria can fuel their survival by scavenging H_2 from the air using specialized hydrogenase enzymes. We propose that this process has a major role in sustaining aerated soil communities by supporting dormant microbial populations during periods of nutrient deprivation. This article provides perspectives on the biology, chemistry, and significance of H_2 scavenging.

THE SINK OF THE GLOBAL HYDROGEN CYCLE

Atmospheric H_2. As the most fundamental element, hydrogen constitutes approximately 75% of the universe by mass. However, the majority of the Earth's H_2 has escaped the atmosphere or become assimilated into inorganic and organic compounds following 4.5 billion years of abiotic and biotic processes (3). The present-day concentration of H_2 in the Earth's troposphere is approximately 530 ppb (parts per billion by volume [ppbv]) (~400 pM in aqueous solution) (1, 4). As recently reviewed (3, 5), extensive geochemical studies have shown that the distribution of this gas varies only weakly temporally and spatially in the troposphere. There is no evidence for a continuous trend in the tropospheric H_2 burden, since the beginning of monitoring programs in the 1980s, as the H_2-oxidizing and H_2-producing processes that affect the net abundance of atmospheric H_2 are approximately balanced. It is unknown whether the atmospheric burden of H_2 would increase as a result of transition to a H_2-based fuel economy (6, 7).

Global hydrogen cycle. H_2 is rapidly turned over in the present-day atmosphere, with the average lifetime of an emitted H_2 molecule predicted to be between 1.4 and 2.1 years (3, 4, 8). Like other biogeochemical cycles, this turnover depends on biological, geochemical, and anthropogenic contributions. Using both "bottom-up" and "top-down" approaches, a wealth of data has been collected on the relative and absolute contributions each process makes to this turnover (Table 1). While there are inevitably large margins of errors both within and between data sets, a consensus that the main sources of H_2 are geochemical and anthropogenic has emerged, whereas the main sink is the biological consumption of H_2 in soil ecosystems. It is estimated that each year, 56 to 88 teragrams of H_2 are taken up by soils globally; hence, this process is predicted to account for three quarters of the net tropospheric H_2 consumed each year (3–5, 8, 9). Consistently, because of the greater landmass of the Northern Hemisphere, the atmospheric partial pressure of H_2 is somewhat higher in the Southern Hemisphere than in the Northern Hemisphere (3).

There are numerous microbial processes that depend on H_2 production and consumption, but these processes make only minor contributions to the global H_2 cycle (Table 1) (5, 10). The

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TABLE 1 Estimated global budget of atmospheric H_2 from three different studies

Process or parameter	Value for process or parameter found by the following study[a]:		
	Novelli et al. (4)	Rhee et al. (8)	Ehhalt and Rohrer (3)
Sources			
Fossil fuel combustion (Tg yr^{-1})	15 ± 10	15 ± 6	11 ± 4
Biomass burning (Tg yr^{-1})	16 ± 5	16 ± 3	15 ± 6
Photochemical hydrocarbon oxidation (Tg yr^{-1})	40 ± 16	64 ± 12	41 ± 11
Biological nitrogen fixation (Tg yr^{-1})	6 ± 3	12 ± 10	9 ± 5
Total (Tg yr^{-1})	77 ± 16	107 ± 15	76 ± 14
Sinks			
Hydroxyl radical oxidation (Tg yr^{-1})	19 ± 5	19 ± 3	19 ± 5
Biological soil uptake (Tg yr^{-1})	56 ± 41	88 ± 11	60 ± 25
Total (Tg yr^{-1})	75 ± 41	107 ± 11	79 ± 25
Tropospheric burden (Tg)	155	150	155
Tropospheric lifetime (yr)	2.1	1.4	2.0

[a] The estimated strength of the sources and sinks of H_2 in teragrams per year, as well as its predicted global burden and lifetime of tropospheric H_2, is shown. In 1999, Novelli and colleagues (4) analyzed the budget using a "bottom-up" approach by estimating source and sink terms separately based on globally averaged yields. In 2005, Rhee and colleagues (8) took a "top-down" approach by using seasonal variation of the deuterium budget of tropospheric H_2 to predict the strength of soil uptake. In 2009, Ehhalt and Rohrer (3) based their estimations on a critical, thorough evaluation of the existing literature.

majority of H_2-oxidizing bacteria can only recycle the high concentrations of H_2 produced by biological or geothermal processes, as their threshold for H_2 (i.e., the minimum concentration of H_2 they can oxidize) exceeds the atmospheric concentration of the gas (11–15). In addition, H_2 produced by biological processes (e.g., nitrogen fixation, fermentation) is generally recycled by such H_2-oxidizing organisms (through both intraspecies and interspecies H_2 transfer) without escaping into the atmosphere (5, 10). Hence, atmospheric H_2 scavenging is the only biological process that has a dominant influence on the H_2 cycle.

Atmospheric H_2 oxidation by soil ecosystems was first shown 40 years ago (1, 2). Gas chromatography and tritium exchange measurements demonstrated that whole soils take up H_2 in a biphasic manner, harboring both fast-acting, low-affinity ($K_m >$ 800 nM) activities and slow-acting, high-affinity ($K_m <$ 70 nM) activities. It is now recognized that some [NiFe]-hydrogenases—a family of H_2-metabolizing enzymes formally divided into five phylogenetically and functionally distinct classes (groups 1 to 5) (16, 17)—are responsible for this uptake. It is likely that the low-affinity H_2-recycling *Proteobacteria* (e.g., *Paracoccus denitrificans* with an enzyme with a K_m of 1.1 μM) are primarily responsible for the low-affinity process; they primarily use group 1 [NiFe]-hydrogenases (17), a widespread class of membrane-bound H_2-oxidizing enzymes (17), to recycle the relatively high levels of H_2 produced by biological and geothermal processes (10–12, 18). The organisms and enzymes responsible for the high-affinity process of atmospheric H_2 oxidation remained elusive until recently, and they are the subject of this minireview.

Isolation and identification of hydrogen scavengers. High-affinity H_2 oxidation in soils is heat sensitive, O_2 dependent, and antimicrobial susceptible, suggesting a microbial origin. The process also follows first-order Michaelis-Menten kinetics, consistent with an enzymatic process (2, 18, 19). However, multiple attempts to isolate pure cultures of high-affinity H_2-oxidizing bacteria were unsuccessful (Ralf Conrad, unpublished data). In addition, pure cultures of well-characterized H_2-oxidizing *Proteobacteria* harbored only low-affinity hydrogenases that consumed H_2 well above the atmospheric range (11–15). It was therefore assumed for a number of years that "abiontic hydrogenases" (cell-free hydrogenases embedded in soil) were primarily responsible for the uptake of H_2 by soil systems (10). While a cell-free hydrogenase could be extracted from forest soils, less than 2% of the original activity was recovered (20). It was later confirmed that abiontic hydrogenases make only a marginal contribution to high-affinity H_2 oxidation activity observed in soils and probably originate from lysed high-affinity H_2-oxidizing bacteria (21).

The determinants of atmospheric H_2 scavenging became clearer in 2008 with the isolation of the first high-affinity H_2 oxidizer, *Streptomyces* sp. strain PCB7. Constant et al. (22) enriched for high-affinity H_2 oxidizers using a dynamic microcosm chamber and identified a sporulating streptomycete that could take up H_2 at picomolar concentrations (~50 to 400 pM). It was subsequently verified that the H_2 oxidation kinetics of nonaxenic soil was similar to that of sterile soil amended with *Streptomyces* sp. PCB7 (21); the extent of high-affinity hydrogenase activity was proportional to the abundance of H_2-oxidizing bacteria in soil. Subsequent soil surveys identified six more high-affinity *Streptomyces* species, suggesting that H_2 scavenging is a common trait among streptomycetes (16). Pure culture studies revealed that the model actinomycetes *Streptomyces avermitilis* (16), *Mycobacterium smegmatis* (23), and *Rhodococcus equi* (24) are also capable of scavenging atmospheric H_2. In pure cultures, such organisms can oxidize tropospheric H_2 given their high affinity (K_m of ~50 nM) and low threshold (~50 pM) for the substrate (16, 22). Genetic analyses have since confirmed that a novel class of enzymes encoded by these organisms, the group 5 [NiFe]-hydrogenases, are primarily responsible for the oxidation of atmospheric H_2 (23).

DETERMINANTS OF HYDROGEN SCAVENGING

Distribution and evolution of group 5 [NiFe]-hydrogenases. All known high-affinity H_2 scavengers carry genes that encode a special group of enzymes, the group 5 [NiFe]-hydrogenases. Genes encoding the large and small subunits of a putative hydrogenase (*hhyLS*) were identified in the genome of *Streptomyces avermitilis* and subsequently detected in numerous other *Streptomyces* isolates (16, 25, 26). Phylogenetic analysis showed that the two genes

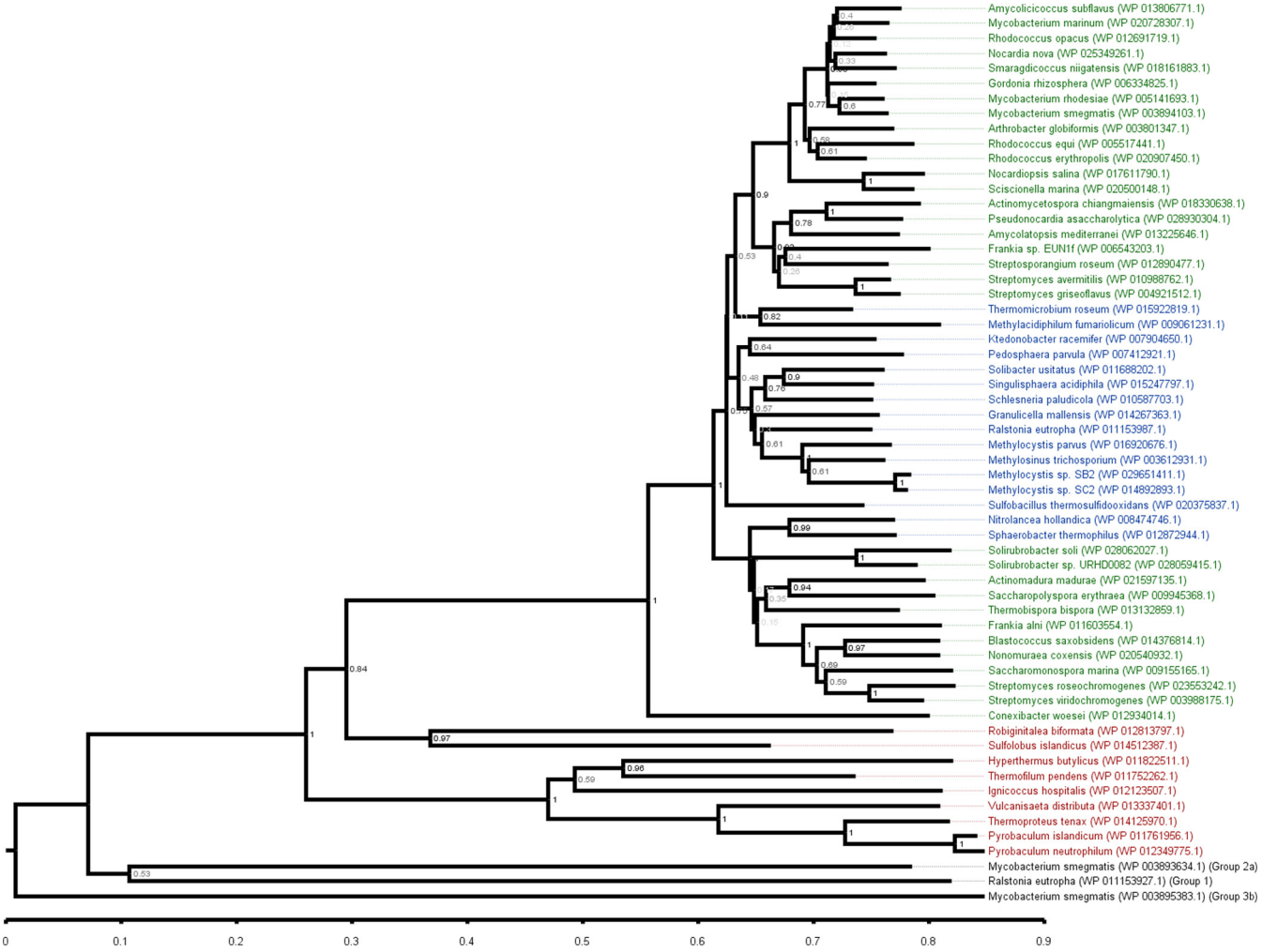

FIG 1 Diversity and distribution of group 5 [NiFe]-hydrogenases. The *hhyL* genes of 60 representative [NiFe]-hydrogenases were aligned and visualized in a bootstrapped neighbor-joining phylogenetic tree. Actinobacterial *hhyL* genes (green), other *hhyL* genes (blue), and crenarchaeotal membrane-bound hydrogenase lineage genes (red) are indicated. The tree is rooted with sequences of the group 2a, group 3b, and oxygen-tolerant group 1 [NiFe]-hydrogenases.

encoding the group 5 [NiFe]-hydrogenases are more closely related to the H_2 uptake [NiFe]-hydrogenases (groups 1 and 2) than the bidirectional and H_2-evolving [NiFe]-hydrogenases (groups 3 and 4). The closest relatives of the enzymes are in fact a deep-branching subclass of membrane-bound [NiFe]-hydrogenases (group 1) encoded in hyperthermophilic archaea (Fig. 1) (16, 25). However, group 5 enzymes have been classified as a distinct group from the group 1 enzymes given that they lack membrane-targeting signal peptides and share low overall sequence identity (17).

BLAST analysis indicates that the group 5 [NiFe]-hydrogenases have a restricted taxonomic distribution, at least among sequenced organisms. To date, genes encoding these enzymes have been identified only in the whole-genome assemblies of aerobic soil and marine bacteria. These enzymes have been widely selected for across soil-dwelling *Actinomycetales* (25), including members of the genera *Mycobacterium*, *Streptomyces*, *Rhodococcus*, *Frankia*, *Amycolatopsis*, and *Saccharomonospora*. They have also been detected in other dominant soil phyla, including *Chloroflexi*, *Acidobacteria*, *Planctomycetes*, *Verrucomicrobia*, methanotrophic *Alphaproteobacteria*, and a betaproteobacterium (Fig. 1). While the number of hydrogenases detected in such groups is small, this is likely because the majority of these groups are severely underrepresented compared to the *Actinobacteria* in public databases (25). Ongoing research and expanding genomic information should provide further information on the capacity of these phyla to scavenge atmospheric H_2.

The group 5 [NiFe]-hydrogenases likely diverged from other uptake [NiFe]-hydrogenases early in evolution prior to the divergence of *Archaea* and *Bacteria*. This is consistent with the closest relatives of these enzymes being encoded in genes in thermophilic *Crenarchaeota* (Fig. 1). However, the incongruence of *hhyL* and 16S rRNA gene sequence phylogenies suggests some lateral acquisition of these enzymes (25). Genes encoding factors involved in genetic mobility have consistently been observed in the vicinity of *hhyLS* genes in 10 species (25, 27). Genes encoding this enzyme have also been acquired in the pHG1 megaplasmid of the model aerobic hydrogenotroph *Ralstonia eutropha* (also known as *Cupriavidus necator*) (28), likely through horizontal gene transfer, but the hydrogenase does not yet appear to have acquired full functionality in this betaproteobacterial host (29).

Structure and mechanism of the group 5 [NiFe]-hydrogenase. A preliminary 2.8-Å resolution crystal structure of the

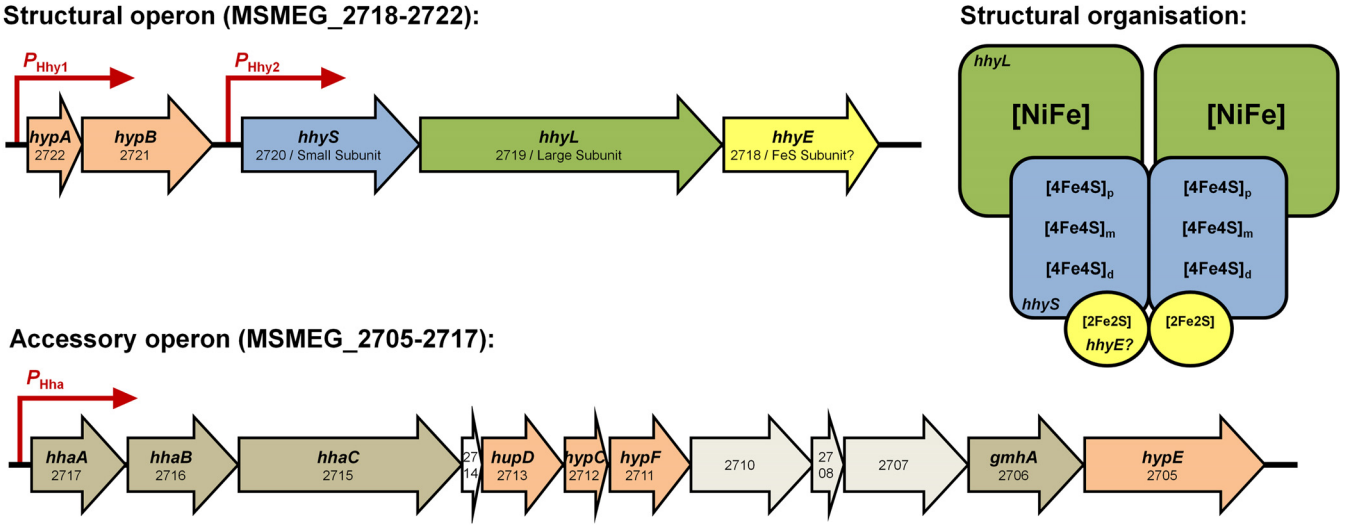

FIG 2 Components of the group 5 [NiFe]-hydrogenase of *Mycobacterium smegmatis*. RT-PCR analysis has clarified that *M. smegmatis* encodes a five-gene structural operon (MSMEG_2718-2722) and a larger accessory/maturation operon (MSMEG_2705-2717) (27, 32). In *Ralstonia eutropha*, the purified enzyme forms a homodimer (30). On the basis of these findings, we predict that the group 5 [NiFe]-hydrogenase of *M. smegmatis* also forms a $(HhyLS)_2$ structure. Encoding a predicted [2Fe2S] cluster, HhyE is likely to accept single electrons and potentially serves as an electron transfer protein for respiration and reductive metabolic processes. The predicted functions of the gene products are indicated by color coding as follows: green for the large subunit, blue for the small subunit, yellow for electron transfer protein, orange for maturation proteins, dark gray for conserved hypothetical proteins, and light gray for hypothetical proteins.

low-affinity group 5 [NiFe]-hydrogenase from *Ralstonia eutropha* provides a revealing insight into structure-function relationships of this group of enzymes (29, 30). Like all known [NiFe]-hydrogenases, this enzyme minimally comprises a closely associated large subunit and a small subunit. The large subunit contains a standard [NiFe]-center (ligated by a carbonyl, two cyanides, and four cysteine residues) that forms the catalytic site for H_2 oxidation. The small subunit contains three iron-sulfur clusters: 3Cys1His$[4Fe4S]_{distal}$, 4Cys$[4Fe4S]_{medial}$, and 3Cys1Asp$[4Fe4S]_{proximal}$ (30). Though the number of clusters is the same as for the well-described group 1 [NiFe]-hydrogenases, the ligands of the proximal cluster and the configuration of the medial cluster differ (31). The metal content and cofactor structures observed in the structure are consistent with those derived from spectroscopic studies (29, 30).

The *R. eutropha* enzyme forms a homodimer, as a result of extensive interactions between the C-terminal α-helical extensions of each small subunit (29, 30). The structure predicts that each monomer forms an electron transport chain, with electrons being tunneled from the catalytic center via proximal and medial clusters to the distal cluster. The still-unidentified physiological electron acceptor is predicted to bind within the vicinity of the distal cluster, perhaps in a binding pocket identified in the crystal structure. Though the catalytic centers between monomers are distant, the distal clusters converge on each other and are sufficiently close to each other in the structure (11.5 Å) that electron transfer could theoretically occur between monomers; hence, it is likely that homodimer formation serves a functional role in addition to a structural one (30).

Maturation and accessory proteins. In *Mycobacterium smegmatis*, reverse transcription-PCR (RT-PCR) analysis under inducing conditions revealed that genes encoding the group 5 [NiFe]-hydrogenases are clustered into two adjacent operons: a structural operon (MSMEG_2718-2722) and an accessory operon (MSMEG_2705-2717) (Fig. 2) (32). The structural operon includes genes encoding the large and small subunits of the enzyme, as well as the nickel insertase HypA, nickel chelator HypB, and a putative [2Fe2S] protein (27). We propose that the [2Fe2S] protein (tentatively annotated as *hhyE*) serves as the immediate electron acceptor for the hydrogenase (23), though this has yet to be verified biochemically or genetically. The accessory operon appears to principally encode maturation and accessory proteins required for production of a functional hydrogenase, including the pleiotropic proteins involved in the biosynthesis of the Fe-$(CN^-)_2$-(CO) center and a putative endopeptidase required to specifically cleave the C terminus of the large subunit. The operon also encodes several hypothetical proteins, including three proteins that are highly conserved and specific to *hhy*-encoding species (herein referred to as *hhaABC* for high-affinity hydrogenase associated proteins); BLAST and COG (clusters of orthologous groups of proteins) analyses provide no clues to their functions (32).

Comparative genomics reveal that the *hhyLSE*, *hhaABC*, and *hypABCDEF* genes are highly conserved across organisms encoding group 5 [NiFe]-hydrogenases. Hence, they are likely to be important determinants for the structure and maturation of the group 5 [NiFe]-hydrogenases. Two other genes show a mosaic distribution among *hhyLS*-encoding organisms, namely, *hypX* and *gmhA*. It has been hypothesized that *hypX* is involved in the maturation of the hydrogenases in *R. eutropha*, where it may serve to protect against oxidative damage (33); however, its absence in *M. smegmatis* and *R. equi* shows that it is dispensable for aerobic H_2 scavenging at least in some organisms (25). Associated with hydrogenases in *M. smegmatis* and *R. eutropha*, but absent in many streptomycetes, the *gmhA* gene is predicted, somewhat perplexingly, to encode a phosphoheptose isomerase (23). In *M. smegmatis*, the structural operon is more strongly induced than the accessory operon; this is consistent with accessory and maturation proteins being required at substoichiometric levels (27, 34).

Membrane localization. Staining of *M. smegmatis* fractions for uptake hydrogenase activity confirmed that the native group 5 [NiFe]-hydrogenase is associated with the membrane (23). However, the homologous enzyme from *R. eutropha* could be purified directly from the cytoplasm (29). The reason for this discrepancy has not been resolved, but it may be physiologically relevant. The *R. eutropha* enzyme behaves significantly differently from the *M. smegmatis* and *Streptomyces* sp. PCB7 enzymes on native polyacrylamide gels, running as two, rather than three, bands (23, 30). It is possible that actinobacterial hydrogenases have different cellular interactions that may contribute to their higher affinities. As no signal peptides or transmembrane helices have been predicted in their primary sequences, it is probable that the enzymes associate with the cytoplasmic side of the membrane through protein-protein interactions or lipid anchors (23).

PROPERTIES OF GROUP 5 [NiFe]-HYDROGENASES

High-affinity H_2 oxidation. The affinities of group 5 [NiFe]-hydrogenases are generally 20-fold higher than other uptake hydrogenases. The apparent Michaelis constants of the enzymes from *M. smegmatis* (23), *S. avermitilis* (16), and *Streptomyces* sp. PCB7 (22) are between 30 and 60 nM. However, *in vivo* and *in vitro* studies have shown that the affinities of group 5 [NiFe]-hydrogenases can vary greatly between organisms. The actinobacterium-type hydrogenase (AH) from *R. eutropha* has a relatively high K_m of 760 nM in whole cells and 3.6 μM when purified. While it has the highest affinity of the four uptake hydrogenases of *R. eutropha*, it is still not capable of oxidizing atmospheric H_2 and may not be fully functional (29, 30). Several streptomycetes also exhibited low to medium affinities for H_2 in plate assays, e.g., *Streptomyces scabiei* and *Streptomyces griseoflavus* (16); however, even subtle differences in the culture conditions or physiological states of cells could influence the biosynthesis and behavior of the hydrogenases under these conditions.

The factors that influence the affinities of [NiFe]-hydrogenases are still not fully understood. It has been postulated that differences in the chemistry at the active site may play a role. Studies of the group 1 [NiFe]-hydrogenase of *R. eutropha* (membrane-bound hydrogenase [MBH]) suggest that the residues surrounding the [NiFe] center directly influences affinity for H_2; amino acid substitutions at the second coordination sphere (e.g., Cys81Ala) reduced the K_m of the enzyme up to 20-fold (35). Schäfer (30) has identified three residues on the second coordination sphere that are substituted between the large subunits of MBH and AH, namely, Cys81 to Asp87, Asp117 to Ile83, and Pro529 to Val500; it has been postulated that these residues might influence the geometry and electron density at the catalytic site. This potentially explains why AH has higher affinity for H_2 of the ralstonial hydrogenases, even if it is still incapable of scavenging atmospheric H_2 (29). It is also probable that the 3Cys1Asp[4Fe4S]$_{proximal}$ cluster has a higher redox potential than the 4Cys[4Fe4S]$_{proximal}$ clusters of standard enzymes, which would make oxidation of low partial pressures of H_2 more thermodynamically favorable (30).

Nevertheless, primary-sequence differences are unlikely to be solely responsible for the spectrum of affinities observed within the group 5 [NiFe]-hydrogenases. Multiple-sequence alignments reveal that the large and small subunits of the enzyme are highly conserved, especially in the L1 and L2 motifs encoding the aforementioned residues, and there are no consistent amino acid differences between high-affinity and low-affinity enzymes (23, 27). We recently proposed that the wider interactions of the enzymes with their physiological electron acceptor and the respiratory chain may modulate their affinities (23). In accord with this, it is imperative to test whether the purified enzymes from *Actinobacteria* retain high affinities *in vitro*. This is consistent with the second uptake hydrogenase of *M. smegmatis*, a group 2a [NiFe]-hydrogenase, also having a surprisingly high affinity for H_2 (23). While the *R. eutropha* enzyme provides valuable insight into the structure and function of group 5 enzymes, this low-affinity enzyme is clearly not suitable for resolving the biochemical basis of atmospheric H_2 uptake (29). Parallel studies on the high-affinity hydrogenases of *M. smegmatis* and *S. avermitilis* will help to resolve what determines the affinities of these enzymes.

Oxygen insensitivity. [NiFe] catalytic centers are inherently sensitive to being inactivated by molecular oxygen (O_2). However, group 5 [NiFe]-hydrogenases have found a way to overcome this: a range of *in vitro* and *in vivo* studies indicate that they are completely insensitive to O_2 (16, 23, 29). This contrasts with the group 1 [NiFe]-hydrogenases of *Proteobacteria* which, while also often O_2 dependent (i.e., they rely on the presence of O_2 as the terminal respiratory electron acceptor), are merely O_2 tolerant; the active sites on the large subunits are oxidized to inactive states (Ni^{III}-B) in the presence of O_2, but they can be rapidly reduced back to their catalytically active states (Ni^{II}-SI) through reversed electron transfer (36–38). Initial spectroscopic studies did not identify any O_2-inactivated states equivalent to Ni^{III}-B in purified AH from *R. eutropha* (30, 31). Furthermore, group 5 [NiFe]-hydrogenases maintained full activity even in the presence of 70% O_2 both *in vivo* and *in vitro* (23, 29).

Two major hypotheses have been put forth about the molecular mechanisms for O_2 insensitivity of the group 5 [NiFe]-hydrogenases. Inspired by landmark studies of the O_2-tolerant [NiFe]-hydrogenases (36, 37), it has been proposed that the nonstandard iron-sulfur clusters could contribute to O_2 tolerance, perhaps through facilitating rapid reverse electron transfer to an oxidized active site (29, 30). It is predicted that 3Cys1Asp[4Fe4S]$_{proximal}$ cluster has a higher potential and that 4Cys[4Fe4S]$_{medial}$ has a lower potential compared to the potentials of the clusters of group 1 [NiFe]-hydrogenases (30, 39). Substitutions of the Asp35 residue of AH large subunit consistently made the group 5 [NiFe]-hydrogenase more O_2 susceptible (30). A distinct but not necessarily competing hypothesis is that group 5 [NiFe]-hydrogenases sterically restrict access of O_2 to the active site. Mutagenesis studies have established that residues lining the substrate channel are important for O_2 tolerance of certain hydrogenases (40, 41). The crystal structure of AH reveals that the gas diffusion channel is significantly narrower than that of the group 1 [NiFe]-hydrogenase, which may contribute to differences in O_2 susceptibility (30).

Thermostability. A further unusual property of the group 5 [NiFe]-hydrogenase is its thermostability. Atmospheric H_2 oxidation by soil samples has been observed at temperatures ranging from −4°C to 60°C (3, 42, 43). The enzyme purified from *R. eutropha* has a half-life of 80 min at 60°C and retains catalytic activity even at 80°C with a half-life of 3.5 min (30). The half-life of H_2 oxidation activity is significantly shorter in whole soils at such temperatures, perhaps because of killing of the H_2-scavenging organisms (44). While phylogenetics suggest that the enzyme has a thermophilic origin (Fig. 1), it is possible that environmental pressure has continued to select for this trait; after all, thermostability

could contribute to the well-characterized heat resistance of certain mesophilic actinobacteria, especially sporulating streptomycetes (45). Group 5 [NiFe]-hydrogenases have also been identified in the genomes of several thermophilic acidobacteria and chloroflexi found in geothermal soils (Matthew Stott, GNS Science, personal communication). While the biochemical basis of this thermostability is not understood, Schäfer (30) has proposed that homodimer formation could be a contributing factor.

PHYSIOLOGICAL ROLE OF HYDROGEN SCAVENGING

An input to the respiratory chain. Much evidence suggests that group 5 [NiFe]-hydrogenases are energy-conserving enzymes linked to aerobic respiratory chains. In whole soils, O_2 is either a prerequisite for H_2 scavenging (46) or an extremely strong stimulant (18). In isolated *Actinobacteria*, O_2 is also required for oxidation of atmospheric H_2 (16, 22, 23). Controlled amperometric and chromatographic measurements showed that H_2 oxidation strictly depends on the presence of O_2 in batch cultures of *M. smegmatis* (23, 27). H_2 oxidation can also be inhibited in *R. eutropha* through addition of the terminal oxidase inhibitor potassium cyanide (30). A link to the respiratory chain is also provoked by studies of the regulation, localization, and phenotypes associated with these enzymes in *M. smegmatis* (23, 27, 34, 47, 48). The combustion of H_2 by O_2 (the "Knallgas" reaction) is one of the most energetically favorable biological reactions under standard biological conditions ($E^{\circ\prime} = +1.24$ V) (49). Hence, H_2 scavengers can maximize the amount of energy they can derive through electron transfer between two ubiquitous atmospheric gases. Given the redox potential of the $2H^+/H_2$ couple is higher at low partial pressure of H_2 (pH_2), low-potential oxidants are unlikely to stimulate atmospheric H_2 oxidation. Atmospheric H_2 oxidation could be stimulated only in anoxic soils with artificial electron acceptors with high redox potentials, i.e., above +80 mV (18).

Mixotrophic growth. To resolve the physiological role of H_2 scavenging, markerless deletions of the group 5 and group 2a [NiFe]-hydrogenases have been constructed in *M. smegmatis*. Gas chromatography studies have shown that both enzymes are capable of scavenging atmospheric H_2 in this organism (16, 22, 23). The wild-type organism appears to be incapable of growing chemolithoautotrophically using H_2 as the sole electron donor (27). However, the growth rate and yields of the deletion strains were significantly reduced compared to those of the wild type during growth on organic carbon sources under a range of conditions. This indicates that *M. smegmatis* preferentially grows mixotrophically by cooxidizing organic electron donors and atmospheric H_2 (27, 34, 48). Phenotypic, transcriptome, metabolome, and energetic studies are consistent with H_2 scavenging providing a source of reductant during growth (48). Inhibitor studies have also substantiated a link between central carbon metabolism and H_2 metabolism in this organism (Kiel Hards, unpublished data). The group 5 [NiFe]-hydrogenase of *R. eutropha* is neither required nor sufficient for chemolithoautotrophic growth. Consistent with its low synthesis level and activity, the enzyme also appears to be dispensable for mixotrophic growth (30).

Electron input during energy limitation. Recent studies have suggested that the physiological role of H_2 scavenging is to input electrons into the respiratory chain during energy limitation. Batch and continuous culture experiments showed that the two H_2-scavenging enzymes of *M. smegmatis* were most synthesized and active when the organism is starved for its preferred organic carbon sources (23, 27, 32, 34). Genes encoding the structural components of the group 5 [NiFe]-hydrogenase and group 2a [NiFe]-hydrogenase were induced 30-fold and 6-fold, respectively, during slow versus fast growth (27, 34). Their upregulation was concurrent with the downregulation of primary dehydrogenases (34), suggesting that oxidation of atmospheric H_2 partly compensates for the reduced oxidation of organic electron donors. When these enzymes were deleted, we observed a 40% reduction in the viability of the mutant strains during their adaptation to and survival of energy starvation in continuous and batch culture systems (34, 48).

H_2 scavenging is intimately linked to the life cycle of *Actinobacteria*. Several groups have shown that expression and activity of the group 5 [NiFe]-hydrogenases peaks after the formation of aerial hyphae in sporulating streptomycetes and that spores appear to be primarily or solely responsible for H_2 scavenging in such organisms (16, 22, 24). *R. equi*, which is a nonsporulating persister like *M. smegmatis*, consumed atmospheric H_2 only in late exponential and stationary phase (24). It is less clear whether the *R. eutropha* enzyme has a physiological role, given that its expression and activity are barely detectable (29), but consistent with group 5 [NiFe]-hydrogenases being selected for a role in energy limitation, expression of the genes encoding AH increased 50-fold when cultures were grown on glycerol (a poor substrate in this organism) compared to fructose (30). More phenotypic studies are clearly needed on the hydrogenases of such organisms to better understand the basis of differential regulation.

While organic electron donors are often sparse in soil ecosystems, H_2 is a dependable fuel source. After all, it is readily diffusible and hence energy-consuming active transport processes are not required to harness it. Furthermore, it is present in unlimited amounts at a constant, albeit trace, concentration throughout the troposphere. Hence, scavenging atmospheric H_2 could be a useful strategy for the survival of a range of soil organisms. Though developmentally and morphologically distinct, the persistent cells of *Mycobacterium* and the spores of *Streptomyces* are both produced in response to energy limitation and require minimal energy input to remain viable (45). We propose that scavenging of atmospheric H_2 provides a proportion of these cells and spores sufficient electron input for long-term persistence. Aided by a physical association with the membrane, high-affinity hydrogenases appear to couple the oxidation of atmospheric H_2 to reduction of ambient O_2 by liberating electrons to the aerobic respiratory chain (Fig. 3). This flux may create sufficient proton motive force for cells and spores to persist in the absence of growth. The insensitivity of high-affinity hydrogenases to environmental stresses, such as temperature, might also ensure that energy generation can occur even under deleterious conditions. It is also noteworthy that pH_2 does not significantly influence the synthesis levels of high-affinity hydrogenases, in contrast to those of low-affinity hydrogenases in soil organisms. This likely reflects that the atmospheric substrate will always be present at sufficient concentrations for catalysis in well-aerated soils (50).

Role in oxygen limitation. H_2 cycling and scavenging are also important for *M. smegmatis* to combat reductive stress during hypoxia. Under this condition, the organism can switch rapidly between three modes of energy conservation, namely, aerobic H_2 respiration, anaerobic H_2 respiration, and fermentative H_2 production, depending on the availability of electron acceptors (47). Batch and continuous culture studies show that the organism in-

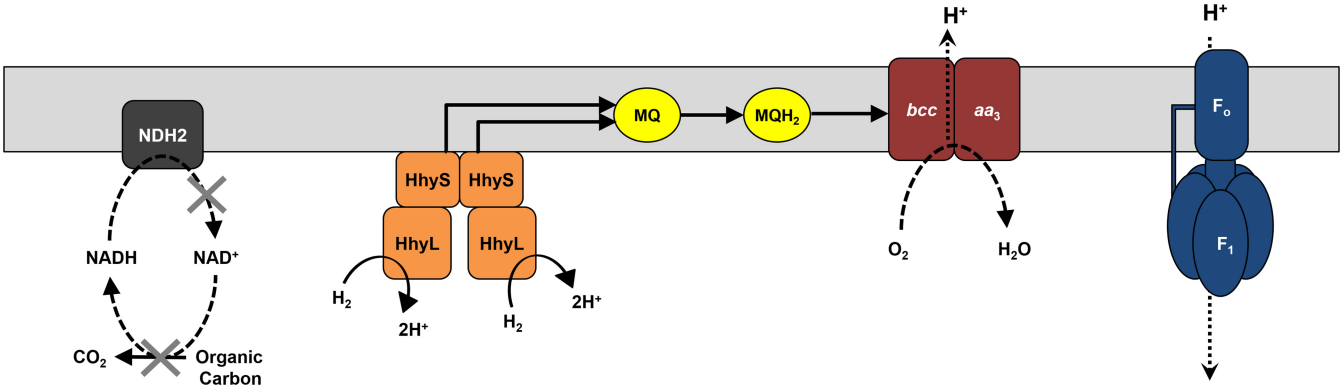

FIG 3 Proposed physiological role of group 5 [NiFe]-hydrogenases during energy starvation in *Mycobacterium smegmatis*. When organic electron donors are limiting, primary dehydrogenases are downregulated in favor of uptake hydrogenases (27, 34). Oxidation of atmospheric H_2 by the group 5 [NiFe]-hydrogenase HhyLS (orange) leads to input of electrons into the respiratory chain (yellow) and proton translocation mediated by the terminal oxidase (red). This generates sufficient proton motive force to allow ATP synthesis by ATPase (blue) to sustain long-term survival. Solid arrows depict electron flow. NDH2, type II NAD(P)H:quinone oxidoreductases; MQ, menaquinone; MQH_2, menaquinol.

duces all three of its [NiFe]-hydrogenases during O_2 deprivation (27, 34, 47). While the group 3b [NiFe]-hydrogenase is responsible for H_2 evolution, the group 2a [NiFe]-hydrogenase can recycle this H_2 and consume it down to atmospheric levels under this condition. The group 5 [NiFe]-hydrogenase is also induced during hypoxia, but its catalytic activity could not be unambiguously observed (47). Consistent with a central role in the hypoxic response, the redox- and O_2-responsive regulator DosR (51) induces expression of the group 5 and group 3b [NiFe]-hydrogenases under this condition (27, 47). Mutants devoid of these hydrogenases or their regulator have lower NAD^+/NADH ratios, impaired adaptation to hypoxia, and a 10-fold reduction in long-term viability during O_2 deprivation (47). No studies have investigated whether the high-affinity hydrogenases of streptomycetes and other organisms are induced under hypoxia.

FROM CELLS TO COMMUNITIES

Hydrogen scavenging is ubiquitous in soils. Consistent with geochemical observations, the results of microcosm studies suggest that atmospheric H_2 oxidation is ubiquitous in aerated soils. Atmospheric H_2 consumption has been observed in a range of soil types, including but not limited to forest, arable, peatland, and desert ecosystems, as well as diverse climates (21, 44, 52). Whereas *hhyL* copy number varied in such samples from 10^7 to 10^8 per gram of soil, the actual H_2 consumption rates varied more significantly and correlated poorly with *hhyL* copy number. This is consistent with environmental and physiological factors influencing the expression and/or activity of the genes (25).

Soil organic content appears to be a dominant influence on the rate of scavenging. The H_2 uptake rate in soils collected in forests, grasslands, and crop fields could be predicted with 80% precision using multiple linear regressions parameterized with the relative abundance of high-affinity H_2-oxidizing bacteria and soil total carbon content (Mondher Khedhiri, INRS, unpublished data). Model residuals are probably explained by the influence of other physical and chemical factors on the H_2 oxidation activity, including the temperature, water content, and pH of the soil samples (3, 5). This link is substantiated by other fieldwork: King (53) demonstrated that oxidation of tropospheric H_2 occurred at significantly higher rates in Hawaiian volcanic soils with reduced organic carbon content and respiration. A significant inverse correlation was also observed between H_2 absorption and CO_2 emission during a yearlong sampling in a rural area (5, 54). Nevertheless, vegetation succession in volcanic deposits in Japan showed higher H_2 oxidation rates in forested than unvegetated soil sites (55), and a survey of the literature highlighted higher rates of H_2 uptake in temperate forests than agricultural areas (3). It therefore appears that atmospheric H_2 represents a relevant energy source for soil microbiome both in oligotrophic and copiotrophic environments.

***p*H_2 as a selection pressure for soil communities.** It is well established that the partial pressure of H_2 in soil ecosystems varies by several orders of magnitude across time and space due to biological processes. Whereas atmospheric H_2 permeates all soils, H_2 is supersaturated in the vicinity of leguminous soils and waterlogged soils due to the respective H_2-evolving activities of N_2-fixing rhizobia and fermentative anaerobes (2, 10, 50, 56, 57). H_2 partial pressure also decreases with soil depth from ambient atmospheric concentrations (530 ppbv) at the surface to threshold levels (<50 ppbv) at a depth of about 10 cm, the gradient depending on the soil microbial H_2 consumption activity (52, 58, 59). Extending physiological studies, we hypothesize that high *p*H_2 (e.g., in the vicinity of root nodules) would select for growth of low-affinity H_2-oxidizing bacteria, i.e., *Alpha-*, *Beta-*, and *Gammaproteobacteria* harboring group 1 [NiFe]-hydrogenases. In contrast, atmospheric and subatmospheric concentrations would sustain the survival of high-affinity H_2-oxidizing bacteria, e.g., sporulating streptomycetes and persistent mycobacteria (Table 2). Thus, whereas booms and busts in *p*H_2 could be a selection pressure for copiotrophs, atmospheric H_2 could provide a dependable lifeline for oligotrophs (32). We hypothesize that the rates, affinities, and efficiencies of H_2 consumption in soil environments could also be a selector for growth and survival.

Several studies have demonstrated that exposure of soils to different partial pressures of H_2 can influence microbial community structure (15, 60, 61). Exposure of agricultural soil microcosms to high *p*H_2 stimulated CO_2 fixation and enriched for H_2-oxidizing *Beta-* and *Gammaproteobacteria* (60). In contrast, exposure of soils to a moderate *p*H_2 produced a shift in the soil bacterial community that was reproducible in both microcosms

TABLE 2 Apparent whole-cell affinities for H_2 among H_2-oxidizing soil bacteria[a]

Organism	Apparent K_m (nM)	[NiFe]-hydrogenase group(s)	Reference
High-affinity hydrogen oxidizers			
Mycobacterium smegmatis	110	2a, 3b, 5	23
Rhodococcus equi	110	3b, 5	24
Streptomyces avermitilis	40	5	16
Streptomyces sp. strain AP1	30	5	16
Streptomyces sp. strain PCB7	50	5	22
High-affinity whole-soil activity	40	5	46
Low-affinity hydrogen oxidizers			
Bradyrhizobium japonicum	800	1	14
Desulfovibrio sp. strain G11	1,100	1, 4	72
Ralstonia eutropha	3,600	1, 2b, 3b, 5	29
Methanospirillum hungatei	5,000	3a, 4	72
Paracoccus denitrificans	1,100	1, 2b	13
Low-affinity whole-soil activity	1,300	1	46
Individual enzymes			
Mycobacterium smegmatis group 5 [NiFe]-hydrogenase	50	5	23
Mycobacterium smegmatis group 2a [NiFe]-hydrogenase	180	2a	23
Ralstonia eutropha group 5 [NiFe]-hydrogenase	760	5	30

[a] Whereas many *Proteobacteria* consume biologically produced H_2 using group 1 [NiFe]-hydrogenases (13, 14, 72), some *Actinobacteria* can scavenge atmospheric H_2 using group 5 [NiFe]-hydrogenases (16, 22–24). The kinetics of these two activities correspond to the biphasic uptake observed in soils (46, 73). Genetic deletions show that, whereas the group 5 [NiFe]-hydrogenase of *Mycobacterium smegmatis* is a high-affinity enzyme (23), its homolog in *Ralstonia eutropha* has a low affinity for H_2 (30). All affinities were determined from whole-cell studies; to date, no high-affinity hydrogenase has been purified.

and the field; the relative abundance of ribotypes corresponding to soil actinomycetes, specifically *Pseudonocardia*, *Mycobacterium*, and *Streptomyces* species, increased (61). Hence, moderate pH_2 might select for the high-affinity group 5 and group 2a [NiFe]-hydrogenases encoded by these genera over the low-affinity proteobacterial group 1 [NiFe]-hydrogenases (16, 23, 62). Several other studies have suggested that low H_2 concentrations also enrich for actinomycetes (63) and *hhyL* genes (64). Ongoing studies are further analyzing how pH_2 influences bacterial community structure and hydrogenase expression.

Fuelling a dormant population. In light of these physiological and ecological studies, we propose that atmospheric H_2 scavenging is important for sustaining the survival of microbes in energy-starved soils. It is predicted that 0.1 to 5% of the total microbial biomass in soils is active, with the remainder in a gradient of dormant states (65). Atmospheric H_2 scavenging may be a particularly effective mechanism to sustain a proportion of these cells. While nonreplicating cells have drastically reduced energy expenditure, they cannot be metabolically inactive: energy input is still necessary for basic cell maintenance, environmental sensing, and especially in the case of sporulators, structural changes (66). We propose that atmospheric H_2 scavenging is a particularly dependable and robust process to generate energy for persisters. The stress tolerance of the group 5 [NiFe]-hydrogenase (29), combined with the ubiquity of atmospheric H_2 and the abundance of the electron acceptor O_2 (3), would enable scavenging to occur in a range of chemically and physically challenging environments. This may in turn contribute to the relative stability of microbial community structures in soils (66).

Atmospheric H_2 scavenging is likely to sustain a significant population of dormant cells. The geochemical profile of atmospheric H_2, including its regular distribution, high uptake (56 to 88 Tg $year^{-1}$), and short lifetime (1.4 to 2.1 $year^{-1}$), suggest that the microbial sink is highly active (3, 4, 8). Based on the rates of atmospheric H_2 uptake observed in whole soils and streptomyces cultures, theoretical calculations have estimated that H_2 scavenging could sustain the maintenance energy required for the survival of a maximum population of 10^6 to 10^7 H_2-oxidizing bacteria per gram of soil (16, 67). Though atmospheric H_2 scavenging has so far been detected only in *Actinobacteria*, *hhyLS* genes have been identified in six of the nine dominant bacterial phyla in soil (68). It is especially interesting that these genes are found in the genomes of *Acidobacteria*, *Chloroflexi*, *Planctomycetes*, and *Verrucomicrobia* (Fig. 1), the importance and characteristics of which are only starting to be understood (68, 69). That *hhyLS* is found in these organisms, while also being expressed and active in streptomyces spores and mycobacterial persisters (16, 23), further suggests that atmospheric H_2 scavenging could fuel a significant quantity of dormant cells. Consistently, it is noteworthy that *Actinobacteria* are among the most abundant taxa in some of the most nutrient-deprived, physically demanding, underdispersed soil ecosystems (70, 71). Could atmospheric H_2 provide the fuel to sustain the development and survival of such communities?

CONCLUSIONS

The findings on atmospheric H_2 scavenging provide a new paradigm for understanding how microorganisms persist under stress-inducing conditions. It is irrefutable that nongrowing cells and even spores require energy to remain viable, but the energy sources that fulfill this need have largely been underlooked. It is probable that microorganisms have adapted to consume a range of exogenous and endogenous substrates to maintain viability. On first inspection, it is surprising that organisms have evolved to consume a substrate available at mere picomolar concentrations. However, the recent studies on the regulation, physiology, and biochemistry of this process provide a rationale for this process. While atmospheric H_2 is insufficient to sustain growth, consumption of this ubiquitous, diffusible trace gas provides a portion of

the energy needed for oligotrophs to survive chemically and physically challenging soil conditions. The group 5 [NiFe]-hydrogenases appear to be well adapted to provide this lifeline. Now that the basics of H_2 scavenging are largely understood, there is much room for more-detailed studies on the biochemistry of high-affinity hydrogenases, the physiological integration of H_2 scavenging, and the importance of this process at the ecosystem level.

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