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lation, and there is therefore little hope of acquiring new resistance alleles.

For generations, the modern dog has served as little more than a culture medium for CTVT. The genome sequence of this living fossil will, together with the discovery of new mutations that control the phenotypes of modern dogs, provide deeper insight into the appearance and perhaps even the behavior of the ancient dog. In addition, the sequence analysis will reveal information about the evolutionary history of the tumor. For exam-

ple, CTVT has evolved methods of fooling the host's immune system, allowing it to establish a colony long enough for transfer between individuals to occur. Elucidation of these mechanisms based on the accumulation and position of mutations may help us understand the means by which pathogens escape host surveillance. This domestic dog tumor provides us with a unique system for answering some intriguing questions, making one wonder what else is hiding in the doghouse.

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BIOCHEMISTRY

Making the H-Cluster from Scratch

Christopher J. Pickett

Many microorganisms use dihydrogen (H_2) as an energy vector between and within cells. This process is underpinned by FeFe and NiFe hydrogenases (1), which reversibly combine protons with electrons to give H_2 at very high rates. An understanding of the chemical mechanism by which they operate may lead to new technology for biological or bio-inspired catalysis (2, 3). Some 15 years ago, crystallographic and spectroscopic studies revealed the essential features of the active site of FeFe hydrogenase, the H-cluster (4). In the H-cluster, a dithiolate-bridged diiron subsite with cyanide and carbon monoxide ligands is linked through a cysteinyl bridge to a 4Fe4S cubane cluster (see the figure). On page 424 of this issue, Kuchenreuther *et al.*

(5) provide insights into the biosynthesis of this unusual structure.

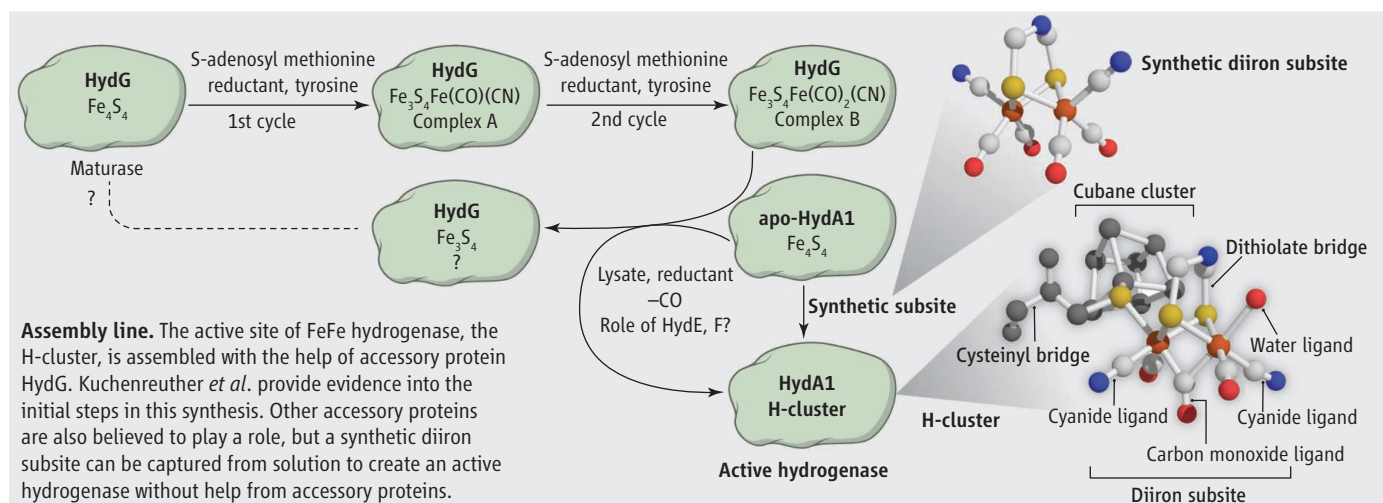
Extensive microbial, biochemical, chemical, and theoretical research is beginning to elucidate how FeFe hydrogenase works (1–4, 6), but it remains unclear how the H-cluster is assembled within the cell (7). In elegant Fourier-transform infrared and electron-nuclear double resonance spectroscopic work involving isotopic labeling, Kuchenreuther *et al.* show that the iron, carbon monoxide, and cyanide components of the subsite in active hydrogenase (HydA1) all originate from chemistry that occurs at a 4Fe4S cubane center of the accessory protein HydG. Transfer of these subsite components—with or without the aid of two other accessory proteins, HydE and HydF—transforms apo-HydA1 (which contains the cluster component of the H-cluster but not the subsite) to the functional hydrogenase HydA1.

Spectroscopic data begin to elucidate the initial steps in the biosynthesis of the hydrogenase active site.

HydG is a radical SAM (S-adenosyl methionine) enzyme that contains two 4Fe4S clusters. Formation of a 5'-deoxyadenosyl radical at one of the clusters leads to H-atom abstraction from tyrosine bound to the other cluster. The resulting dehydroglycine is cleaved to give the Fe(CO)(CN) unit of complex A. This unit is then converted to the Fe(CO)₂(CN) synthon of complex B. The latter is ultimately transferred to apo-HydA1 (see the figure). Kuchenreuther *et al.* suggest that the Fe(CO)(CN) and Fe(CO)₂(CN) moieties of complexes A and B are an integral part of the modified 4Fe4S framework of HydG as Fe₃S₄Fe(CO)₁ or ₂(CN) clusters. This type of structure is unprecedented. However, chemical synthetic results (8) and observations on nitrogenase (9) lend support to the proposed structures.

The work of Kuchenreuther *et al.* extends the portfolio of organometallic radical SAM chemistry and opens up many exciting new

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questions. The formation of complex A requires one tyrosine in the first cycle of the enzyme. But once complex A is formed, the iron site at which this chemistry takes place is at least partially blocked by CO and CN and is electronically very different from that in the resting state of HydG. It remains to be shown how tyrosine is subsequently converted to CO to form complex B (together with a surplus CN⁻) in the second cycle of HydG, and how the Fe(CO)₂(CN) synthon is transferred and coupled to give the dithiolate-bridged subsite in the H-cluster.

By beginning to explain the early stages of H-cluster biosynthesis, the elegant spectroscopic study by Kuchenreuther *et al.* extends our knowledge of how the metallo-

sulfur active sites of a range of enzymes are assembled from simpler building blocks. The accessory proteins HydE, HydF, and HydG are all involved in the *in vivo* assembly of the active H-cluster on HydA (7) and must play a role in some or all of these steps. However, Kuchenreuther *et al.* have previously shown *in vitro* that HydG without HydE or HydF can activate an apo-hydrogenase in the presence of reductant and lysate (10). Given that in the native pathway, all the iron in the subsite moiety originates from HydG (5), it is plausible that a diiron subsite forms from two (or possibly one) HydG synthons, with subsequent release into solution and capture by apo-HydA1. Support for this idea comes from a study showing that a synthetic diiron

subsite (see the figure) (11) can be captured from solution by apo-HydA1 to give an active FeFe hydrogenase without participation of HydE or HydF (12).

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ATMOSPHERIC SCIENCE

Climate Effects of Aerosol-Cloud Interactions

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Aerosols counteract part of the warming effects of greenhouse gases, mostly by increasing the amount of sunlight reflected back to space. However, the ways in which aerosols affect climate through their interaction with clouds are complex and incompletely captured by climate models. As a result, the radiative forcing (that is, the perturbation to Earth's energy budget) caused by human activities is highly uncertain, making it difficult to predict the extent of global warming (1, 2). Recent advances have led to a more detailed understanding of aerosol-cloud interactions and their effects on climate, but further progress is hampered by limited observational capabilities and coarse-resolution climate models.

Recent advances have revealed a much more complicated picture of aerosol-cloud interactions (see the figure) than considered previously. For example, radiative forcing due to aerosol-cloud interactions may be limited by buffering mechanisms that result in compensation between different cloud responses to aerosols (3). Other situations may be hypersensitive to aerosols because

aerosols have become extremely depleted by precipitation (4). In these ultraclean regimes, addition of aerosols can dramatically increase cloud cover, causing a large cooling (5). Another newly appreciated process is aerosol-induced invigoration of deep convective clouds that may transport larger quantities of smaller ice particles to the anvils of such clouds. The higher, colder, and more expansive anvils can lead to warming by emitting less thermal radiation to space (6).

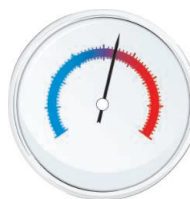
The Intergovernmental Panel on Climate Change's fifth assessment report (2) begins to account for some of these aerosol cloud-mediated effects. Most studies address a subset of known or suspected mechanisms, and they generally cannot separate individual contributions. Yet, this represents advancement with respect to the fourth assessment report (7), which accounted for only one specific effect: the aerosol-induced reduction of cloud drop size and the resultant increasing cloud solar reflectance. It is now clear that the reduced cloud drop size triggers other processes that may induce larger radiative perturbations than the droplet-size effect through mechanisms such as those depicted in the figure (8). The inability to fully quantify these effects increases the uncertainty in the radia-

Advances in satellite observations and model development are needed to disentangle the complex interactions of aerosols and clouds and their effects on climate.

tive forcing of aerosols and clouds. Furthermore, little is known about the unperturbed aerosol level that existed in the preindustrial era. This reference level is very important for estimating the radiative forcing from aerosols (9). Quantification of the reference level requires better quantitative understanding of the natural and anthropogenic emission sources and their interactions.

At fine scales (tens of meters or less), the processes by which aerosols alter the formation and growth of cloud drops and by which drops coalesce into rain are comparatively well understood, as are the ways in which turbulence affects these processes. Less clear is the response of the cloud cover and organization to the loss of water by rainfall. Understand-

ing of the formation of ice and its interactions with liquid droplets is even more limited, mainly due to poor ability to measure the ice-nucleating activity of aerosols and the subsequent ice-forming processes in clouds. Explicit computer simulations of these processes even at the scale of a whole cloud or multicloud system, let alone that of the planet, require hundreds of hours on the most powerful computers available. Modelers must therefore resort to simple parametric representations of these processes



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