this question will be hard to answer. However, current models of DNA substitution usually fit the data poorly (*19*), and a 12S rRNA study (*20*) indicates that the most sophisticated methods of modeling site-to-site variation do not always give the correct tree, leaving open the possibility that these methods may also fail to prevent long branch attraction in 18S rRNA phylogenies of the animal phyla.

The amino acid sequences of proteins may be more immune to the problems of long branch attraction than the nucleotide sequences of 18S rRNA, and protein-coding genes constitute a much larger proportion of the genome than RNA-coding genes. Thus, it is likely that protein sequences will become a major source of data for inferring phylum-level relationships, especially with the growing number of animal genome projects.

Given the probable rapid divergence of most of the animal phyla, the complexities of 18S rRNA sequence evolution, and the problem of taxon sampling, it is difficult to have confidence in 18S rRNA trees in the absence of corroborating evidence. Fortunately, morphological and 18S rRNA phylogenies usually agree in their coarse structure. For example, there is agreement in the basal position of the diploblastic animals (which include jellyfish and corals), the grouping of the echinoderms, the hemichordates, and chordates; and the close relationship of the major protostome phyla such as mollusks, arthropods, and annelids (the "true" worms). But there are frequent minor and sometimes major disagreements, such as in the position of the lophophorate phyla (or even whether they are each other's closest relatives) (*3*). In cases of disagreement, it is an open question as to which (if either) interpretation is correct.

To be confident in our hypotheses of relations among the animal phyla we need to gather more DNA sequences, especially from undersampled phyla; develop better methods of DNA analysis on the basis of more realistic models of DNA evolution (*21*); and develop independent data sets using morphological, developmental, and other molecular data (*4*, *7*) to corroborate or falsify specific hypotheses or to combine in total-evidence analyses (*22*). Work is currently under way on all these fronts, which promise more secure hypotheses of the relationships among the animal phyla and, through them, a better understanding of the causes of major morphological innovation.

References and Notes

1. K. G. Field *et al.*, *Science* **239**, 748 (1988).
2. See http://phylogeny.arizona.edu/tree/eukaryotes/animals/animals.html for earlier references.
3. A.-M. A. Aguinaldo *et al.*, *Nature* **387**, 489 (1997).
4. G. Balavoine, *C. R. Acad. Sci. Ser. III Sci. Vie* **320**, 83 (1997).
5. K. M. Halanych *et al.*, *Science* **267**, 1641 (1995).
6. L. Y. Mackey *et al.*, *J. Mol. Evol.* **42**, 552 (1996).
7. M. E. Siddall, D. S. Martin, D. Bridge, S. S. Desser, D. K. Cone, *J. Parasitol.* **81**, 961 (1995).
8. M. J. Telford and P. W. Holland, *Mol. Biol. Evol.* **10**, 660 (1993).
9. J. M. Turbeville, J. R. Schulz, R. A. Raff, *ibid.* **11**, 648 (1994).
10. S. Carranza, J. Baguñà, M. Riutort, *ibid.* **14**, 485 (1997).
11. C. R. Marshall; *Comp. Sci. Stat.* **29**, 218 (1997).
12. G. Lecointre, H. Philippe, H. L. V. Lê, H. L. Guyader, *Mol. Phylogenet. Evol.* **2**, 205 (1993).
13. J. Felsenstein, *Syst. Zool.* **27**, 401 (1978).
14. M. D. Hendy and D. Penny, *ibid.* **38**, 297 (1989).
15. J. Kim, *ibid.* **45**, 363 (1996).
16. Y. Van de Peer, J.-M. Neefs, P. D. Rijk, R. De Wachter, *J. Mol. Evol.* **37**, 221 (1993).
17. S. Conway Morris, *Curr. Biol.* **7**, R71 (1997).
18. H. Philippe, A. Chenuil, A. Adoutte, *Dev. Suppl.*, 15 (1994).
19. J. P. Huelsenbeck and B. Rannala, *Science* **276**, 227 (1997).
20. J. Sullivan, K. E. Holsinger, C. Simon, *Mol. Biol. Evol.* **12**, 988 (1995).
21. J. Felsenstein and G. A. Churchill, *ibid.* **13**, 93 (1996).
22. A. de Queiroz, M. J. Donoghue, J. Kim, *Annu. Rev. Ecol. Syst.* **26**, 657 (1995).

23. I thank S. Tavaré and D. Erwin for helpful comments. Supported in part by NSF grant EAR-9258045 to C.R.M.

CHEMISTRY

Fixing Nitrogen Any Which Way

G. J. Leigh

This issue of *Science* contains (*1*) a stimulating report by Nishibayashi *et al.* (page 540) on the conversion of dinitrogen to ammonia. This report begins to show a gradual intertwining of many diverse strands of research into dinitrogen reactivity. This is all the more ironic in that the big expansion in nitrogen fixation research during the 1970s and 1980s has moved into reverse now that the direct economic return has been judged to be disappointing.

There are at least four different kinds of reactivity of dinitrogen described in the literature. Not all are fully defined, and some are very far from being mechanistically understood. The oldest in research terms is the Haber synthesis (*2*). This operates at high temperatures and pressures and uses a promoted metallic iron catalyst. The reaction appears to occur by chemisorption of both dihydrogen and dinitrogen on the catalyst, surface, followed by stepwise assembly of ammonia from these atoms. Highly reduced systems, such as a mixture of a metal halide plus an excess of a Grignard reagent, that react with dinitrogen to form ill-defined nitrides have been recognized for many years, but the clean splitting of dinitrogen by a complex to form a nitrido complex has been achieved only recently, by Cummins and his collaborators (*3*). In contrast, splitting of dihydrogen by metal complexes to form metal hydrides has long been known. As yet, no simple coordination compound can perform these two functions simultaneously, which is why metal complexes that are Haber-type catalysts are unknown. Chemists have comforted themselves with the thought that a metal surface can do things that complexes cannot do. In any case, there is little likelihood of developing a Haber catalyst that is as easy to prepare and as stable mechanically and chemically as metallic iron.

Biological catalysis of nitrogen fixation has provoked a great deal of speculation, some of it well founded. The now-characterized iron-molybdenum-sulfur cluster at the heart of the molybdenum nitrogenases (*4*) might appear to be a biological analog of the Haber catalyst, at least as far as the splitting of dinitrogen is concerned. In fact this is unlikely. No metal-sulfur cluster has yet been shown to react with dinitrogen. In any case, the reaction catalyzed by nitrogenases in biological systems fundamentally involves dinitrogen and water (plus an energy input) rather than dinitrogen and dihydrogen (plus an output of energy) as in the Haber process.

It is now generally accepted that the most efficient biological fixation by molybdenum nitrogenases involves the following stoichiometry:

$$N_2 + 8H^+ + 8 \text{ electrons} \rightarrow 2\,NH_3 + H_2 \quad (1)$$

The reasons for the production of dihydrogen are not clear. In addition, it seems that two molecules of adenosine 5′-triphospate (ATP) are hydrolyzed for the transfer of each electron, 16 in all for a single catalytic cycle. However, non-molybdenum nitrogenases exhibit different stoichiometries and that in any case the protein binding the cluster seems to be a necessary component of the nitrogenase system. The isolated cluster cannot fix nitrogen.

Now much of this dogma has been thrown into doubt. Although it was noted

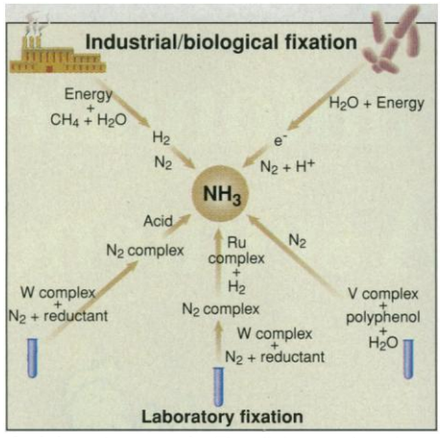
The author is in the School of Chemistry, Physics, and Environmental Science, University of Sussex, Brighton BN1 9QJ, UK. E-mail: kafc1@maila.central.susx.ac.uk

in 1992 (5) that *Streptomyces thermoautotrophicus* is able to fix nitrogen, it now transpires (6) that its nitrogenase, supposedly a molybdenum nitrogenase, is different from all the rest. It uses electrons from carbon monoxide oxidation to reduce dinitrogen. The system depends on dioxygen as an electron transfer agent, and it cannot reduce ethyne to ethene, generally believed to be an invariable property of nitrogenases. Both carbon monoxide and dihydrogen are growth substrates. At best, it uses only four molecules of ATP to turn the complete catalytic cycle rather than 16, so that the equivalence of one electron transferred for each two molecules of ATP hydrolyzed cannot hold. It does not contain an analog of the highly conserved iron protein characteristic of all other nitrogenases, hitherto the only known electron carrier to the nitrogen-fixing clusters. Nevertheless, it contains an as-yet incompletely defined iron-molybdenum-sulfur cluster that may be similar to the "classical" cluster.

The third area of reactivity is that developed by the Soviet school of chemistry, principally that associated with the name of Shilov (7). The unique aspect of this work has been the development of systems that fix nitrogen in an aqueous environment. For example, a mixture of a compound of vanadium(II) with a polyphenol such as catechol fixes nitrogen, but only within a narrow band of pH, well on the alkaline side. The original work has been expanded to mimic biological systems more closely, and it has proved possible to use electrodes to provide the reducing power. Some of these systems are genuinely catalytic, but definitive mechanisms have never been determined. These often heterogeneous systems are unique in their catalytic properties and in their ability to reduce dinitrogen and to function in the presence of water.

The final class of reactivity is that of dinitrogen in a complex with electrophiles, including the proton and organic free radicals. The mechanism of this kind of reaction has been reasonably well established, especially with singly bound, end-on dinitrogen complexes (8). The protonation is stepwise. The electrons flow from the metal ion, which becomes oxidized, into the dinitrogen, which simultaneously binds protons. These two processes maintain an approximate balance of neutrality in the dinitrogen fragment that eventually generates hydrazine or ammonia, or both. This kind of system has never been made truly catalytic, probably because, although a large supply of protons is easily introduced in the form of a mineral acid, the supply of six electrons from a metal ion while retaining the essential integrity of dinitrogen binding and reducing complex is very difficult to achieve.

The use of acidic transition-metal complexes such as $[HCo(CO)_4]$ and $[Ru(C_5H_5)\{(CF_3C_6H_4)_2PCH_2CH_2P(CF_3C_6H_4)_2\}H_2]^+$ rather than a mineral acid to protonate complexed dinitrogen is relatively recent (9). They are not the most easily accessible of protonating agents, and their synthesis from dihydrogen is not necessarily facile. The report by Nishibayashi *et al.* (1) goes some way toward solving this problem. The authors show that the complex $[RuCl(Ph_2PCH_2CH_2CH_2PPh_2)_2]^+$ can react reversibly with dihydrogen, and that the resultant dihydrogen adduct can heterolyze in the presence of the long-known tungsten derivatives $[W(N_2)_2(PMe_2Ph)_4]$ and $[W(N_2)_2(Ph_2PCH_2CH_2PPh_2)_2]$, protonating the dinitrogen to yield ammonia and traces of hydrazine. Evidence is presented to show that hydrazido(2-) species are intermediates.



Arriving at ammonia. The dinitrogen bond can be split by several mechanisms in industry and nature (**top**) and the lab (**bottom**).

In the presence of acetone, azines are produced, which implies that a condensation has taken place between the acetone carbonyl group and an NH_2 group to eliminate water.

The novelty of these findings is twofold. First, dihydrogen is necessary for the reaction. It is therefore tempting to assign a genuine catalytic role to the ruthenium complex. However, there is roughly only one mole equivalent of ruthenium dihydrogen complex present in the equilibrium mixture, and at the best about three such mole equivalents ought to be used in the protonation of coordinated dinitrogen to produce one mole equivalent of ammonia, if the ruthenium complex is the sole source of protons. It would appear that only about one mole of the ruthenium dihydrogen complex is definitely used, because the best ammonia yields before base distillation are about 16%. Much higher yields are obtained after base distillation.

The second novel aspect is a new reactivity of coordinated dinitrogen in forcing a heterolysis on coordinated dihydrogen. The mechanism is not clear, although the result seems to be a reaction very similar to the well-known dinitrogen-protonation reactions cited above. It may be related in general type to the reaction of coordinated dinitrogen with coordinated dihydrogen in a dinuclear zirconium complex reported by Fryzuk *et al.* (10), one difference being that in the present case there are two coordination sites available on different complexes, whereas in the zirconium case there are two sites within the same complex.

The use of dihydrogen as an electron source under mild chemical conditions to reduce dinitrogen seems to bridge a gap between the Haber chemistry and coordinated dinitrogen chemistry. It also raises again the question of whether the final reductant of dinitrogen in the nitrogenases might not also be dihydrogen. Although there is as yet no definitive answer to this question, the properties of the nitrogenase from *S. thermoautotrophicus* do not yet lend much to support the idea. The chemistry of dinitrogen is turning out to be much richer than was ever expected. The major problem in constructing a new chemical catalytic system would seem to be the regeneration of the dinitrogen-binding site once a molecule of dinitrogen has been reduced, and there has been no progress on that front for many years.

Finally, the ability to combine dinitrogen and dihydrogen to produce ammonia under mild conditions is something that chemists have long sought. However, the possibility that the Haber process will be revolutionized and replaced seems remote. Apart from any other considerations, a major cost in the current industrial process is the preparation of dihydrogen. In this particular context, a different reductant might be a more attractive alternative. Nitrogenases use water plus energy, rather than the natural gas plus energy used industrially. An old report (11) finds that methane and water can be used directly to reduce dinitrogen in the presence of a silica-based ruthenium catalyst. This obviates the need to generate dihydrogen independently, and I would still guess that for industrial processes this kind of reaction represents a more likely way forward.

References

1. Y. Nishibayashi *et al.*, *Science* **279**, 540 (1998).
2. J. R. Jennings, Ed., *Catalytic Ammonia Synthesis* (Plenum, New York, 1991).
3. C. E. Laplaza and C. C. Cummins, *Science* **268**, 861 (1995); C. E. Laplaza *et al.*, *J. Am. Chem. Soc.* **118**, 8623 (1996).
4. J. Kim and D. C. Rees, *Biochemistry* **33**, 389 (1994).
5. D. Gakari *et al.*, *J. Bacteriol.* **174**, 6840 (1992).
6. M. Ribbe *et al.*, *J. Biol. Chem.* **272**, 26627 (1997).
7. T. A. Bazhenova and A. E. Shilov, *Coord. Chem. Rev.* **144**, 69 (1995).
8. G. J. Leigh, *Acc. Chem. Res.* **25**, 177 (1992).
9. M. Hidai *et al.*, *Chem. Lett.* (1980), p. 645; G. Jia *et al.*, *Inorg. Chem.* **30**, 594 (1991).
10. M. D. Fryzuk *et al.*, *Science* **275**, 1445 (1997).
11. S. Naito and K. Tamaru, *J. Chem. Soc. Chem. Commun.* (1978), p. 1105.