inevitable that, with a thin icy crust underlain by rock, impact excavation would have brought up rocky material, mixing it with the surface ice: the present ice layer must therefore have formed after the period of heavy bombardment. With a thicker ice (or water and ice) outer shell, impact need not have penetrated to the rocky layers and the effects of impact might have been removed by cold viscous flow in the ice crust. Alternatively, early formed craters might have been eroded by the sputtering effect of charged particles capable of removing tens of metres, or even kilometres, from the surface (Smith *et al. Science* **206**, 927; 1979). A thin icy crust must either have formed after the end of bombardment, or have remained in a liquid state, allowing denser silicate material to settle, until after the bombardment period.

The origin of the linear pattern on Europa is particularly enigmatic. Various proposals have been put forward including crustal expansion owing to freezing of an early ocean, global expansion as a result of dehydration of the interior, and tidal deformation. The complexity of the pattern made up of features of different morphology is difficult to interpret in terms of mechanism and indeed more than one process may have contributed to the surface features. At present, interpretation of the surface of Europa is model-dependent; a situation which is perhaps not surprising on a planet whose geology is unlike anything in our previous experience. □

Finding nitrogen fixation genes

from John E. Beringer

THE enzymic conversion of atmospheric nitrogen to ammonia is carried out only by prokaryotic microorganisms. The structure and properties of the enzyme complex used, nitrogenase, are very similar in all species that have been studied.

Nitrogenase consists of two main components. One is an iron protein, composed of two identical subunits; it binds MgATP and transfers electrons to the other component which reduces the substrate. The second component is a molybdenum-iron enzyme containing four proteins which can be separated into two distinct sub-units, giving the enzyme an $\alpha_2\beta_2$ type of structure. All these proteins are irreversibly denatured by oxygen. A further striking similarity between nitrogenases is that a functional enzyme can be constructed by mixing iron and iron-molybdenum proteins from different genera; for example *Klebsiella* and *Azotobacter*, though some combinations such as *Klebsiella* and *Clostridium* are inactive.

Over the last few years there have been dramatic advances in our understanding of the genetics of nitrogen fixation (Nif). This has been due largely to the fortunate finding that *Klebsiella pneumoniae nif* genes are linked to each other and could be manipulated in *Escherichia coli*. As a result of extensive mutant and complementation studies we know that there are 17 *nif* genes in seven or eight operons. Three genes code for nitrogenase proteins; *nif* H for the iron protein and K and D for the different subunits of the molybdenum-iron enzyme. Functions have yet to be assigned to some of the other genes.

Parallel genetic studies of *nif* genes in other nitrogen fixing species have been restricted by limitations in our ability to do the necessary genetic manipulations or, in the case of the genetically amenable *Rhizobium* species, our inability to obtain nitrogen fixation in pure culture. However, using *Rhizobium meliloti* as an example, Ruvkun and Ausubel in this issue of *Nature* (see page 85) have shown that one can take advantage of the conservation in DNA sequence of *nif* genes to promote site-directed mutagenesis and localized mapping in prokaryotes.

In 1979 Nuti *et al.* (*Nature* **282**, 533) reported that DNA encoding the *K. pneumoniae* iron and molybdenum-iron proteins hybridized with plasmid DNA from *Rhizobium*, though DNA encoding most of the remaining *Klebsiella nif* genes did not hybridize. In January this year Ruvkun and Ausubel (*Proc. natn. Acad. Sci. U.S.A.* **77**, 191) and Mazur, Rice and Haselkorn (*Proc. natn. Acad. Sci. U.S.A.* **77**, 186) reported further evidence for the conservation of *nif* genes. Ruvkun and Ausubel looked at 19 strains of different nitrogen fixing prokaryotes, including Gram-positive and Gram-negative bacteria, cyanobacteria and the actinomycete *Frankia*. All showed DNA hybridization with the fragment of *K. pneumoniae nif* DNA carrying genes K D and H used by Nuti *et al.*, whereas no hybridization was observed with controls representing ten different non-nitrogen fixing species or with DNA carrying the other *nif* genes. Mazur, Rice and Haselkorn confirmed that hybridization could occur with three strains of cyanobacteria representing two different species. They further showed by heteroduplex studies of hybridized DNA that the genes were not contiguous cyanobacteria strains. It is unclear from the published data for *nif* gene hybridization whether or not all three structural *nif* genes (H D and K) hybridize in all these species. Hopefully this will be resolved in the near future.

Another problem which should be resolved soon is the determination of the extent of DNA sequence homology for these genes in different species. The DNA sequences for the *nif* H proteins of *Anabaena*, (Haselkorn pers. comm.) and *Klebsiella* (Ausubel pers. comm.) are now available. It will be interesting to see how highly conserved the sequences of the different genes are and whether or not the data support the idea that the enzyme has been conserved over a long evolutionary period, or that nitrogen fixation is a relatively new function which has been transferred between different bacterial genera.

These DNA hybridization studies have been of very great value for comparative studies of *nif* genes in different families. As described above, they can be used to determine whether *nif* genes are chromosomal or plasmid borne, whether they are linked to each other and as a method for isolating *nif* DNA for studies of its expression in different hosts or for further genetic studies. It is these last two uses of the *nif* hybridization work which are discussed by Ruvken and Ausubel in this issue of *Nature*. They show, as one would expect, that the *K. pneumonia nif* DNA can be used to isolate the corresponding *Rhizobium* genes and introduce them into *Escherichia coli*. Of much greater importance scientifically is the demonstration that this procedure can be used to induce mutations in the *Rhizobium nif* DNA within *E. coli* and then return the defective gene to its original host where recombination can be forced so that the defective gene is introduced into the chromosome replacing the existing functional gene. In this way mutants of *Rhizobium*, or potentially any other nitrogen fixing prokaryote, which are defective in known *nif* genes can be induced.

Once such a mutation has been induced it is possible to re-isolate DNA fragments extending from this region and induce mutations in them. By knowing the extent of the overlap with the original fragment a series of mutations can be induced at known distances from the original mutation by repeated rounds of mutagenesis and replacement of the corresponding host DNA. While the value of this technique for studies of genes linked to a particular *nif* gene is obvious it is also equally valuable for all species where cloned DNA fragments are available and there is a method for re-introducing the mutated DNA into the host. Thus we now have a technique which could be used to prepare maps of chromosomes or plasmids in a wide range of species of micro-organisms. □

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