Nitrogen fixation symposium

Molecular genetics: out of the laboratory into the field

from Allan Downie

THE Fifth International Symposium on Nitrogen Fixation, an interdisciplinary meeting which ranged from the co-ordination chemistry of nitrogenous compounds to the ecology of diazotrophs, was one of a series aimed at bringing together scientists with interests from all areas relating to nitrogen fixation. It is clear that molecular biology techniques are now having a significant impact on the agriculturally important process of biological nitrogen fixation.

The fundamental research carried out on the molecular genetics of nitrogen fixation in *Klebsiella pneumoniae* has been exploited by those analysing nitrogen fixation in bacteria less amenable to genetic manipulation, including, for example, the cyanobacterium *Anabaena* and *Rhizobium*, both of which participate in symbioses important in agriculture.

Several genes involved in nitrogen fixation in *Anabaena* were cloned using DNA homology by Haselkorn *et al.* (University of Chicago). The glutamine synthetase gene, important in the assimilation of fixed nitrogen, appears to have two promoters, one of which is specifically switched on when *Anabaena* is actively fixing nitrogen. Since nitrogen fixation in *Anabaena* occurs within differentiated cells (heterocysts) it is possible that genes involved in nitrogen fixation are regulated in a similar way to genes involved in spore formation in *Bacillus* and it was suggested that there may be σ-factors which determine the specificity of the RNA polymerase for different promoter sequences. The three structural genes for nitrogenase have also been cloned from *Anabaena* and shown to have a different arrangement from those in *K. pneumoniae*, with the *nif*H and D genes adjacent to each other but separate from *nif*K. For reasons that are as yet obscure, *Anabaena* appears to contain a reiteration of (part of) the *nif*H sequence.

The theme of repeated sequences was one of particular interest to those working with *Rhizobium*. Quinto *et al.* (*Nature* **299**, 724; 1982) were the first to report the reiteration of structural nitrogenase genes in *R. phaseoli*. At this meeting the genes were also shown to be present in *R. trifolii* (Shine *et al.*, Australian National University, Canberra) and *R. meliloti* (Better *et al.*, University of California, San Diego). In *R. meliloti* a series of six highly conserved DNA sequences was reported and these appeared to represent transcriptional control regions for genes expressed by *Rhizobium* during symbiotic nitrogen fixation. Nucleotide sequencing revealed that promotor-like sequences were highly conserved and, surprisingly, that there was more than 80 per cent homology over a 160bp region spanning the sites of initiation of transcription. Downstream of one of these promotors an open reading frame was identified corresponding to a second *nif*H(-like) gene. Surprisingly the similarity in sequence ended abruptly and the function (if any) of this partial gene remains to be established.

The nodulation genes from *R. meliloti* (Long *et al.*, Stanford University) *R. leguminosarum* (Downie *et al.*, University of East Anglia) *R. trifolii* (Shine *et al.*, Scott *et al.*, DSIR, New Zealand) and *R. parasponium* (Marvel *et al.* Harvard University) have been cloned and shown to transfer the plant-host-range determinants between different species of *Rhizobium* and even between *Rhizobium* and *Agrobacterium*. Progress in this area of molecular biology is rapid and a detailed knowledge of the genes and their sequences may soon be available; the next step will be to analyse their biological role. One or more of these genes will almost certainly be important the initial stages of the plant-microbe interaction.

The role of plant lectins and bacterial polysaccharides at the early stages of the plant-microbe interaction was an area of lively discussion. Bhuvaneswari *et al.* (C.F. Kettering Research Lab., Yellow Springs) and Solheim (University of Tromso) reported that *R. trifolii* when cultured in the presence of white clover produced a factor which induced branching of the clover root hairs. Thus the mature root hairs (which are not usually susceptible to infection by *Rhizobium)* could be induced to branch, forming a new growing tip which was susceptible to infection. A (partially) purified preparation of the 'branching factor' (consisting mainly of oligosaccharides) was shown to enhance nodulation, particularly in the mature region of the roots where less nodulation usually occurs. Stacey (University of Tennessee) identified a plant-produced component which has a role in the early steps of the *R. japonicum*/soybean symbiosis. Using a mutant of *R. japonicum* which formed few and delayed nodules on soybeans, he found that a component on soybean root exudate could partially reverse the mutant phenotype of the *Rhizobium* strain. The plant-encoded component appeared to be a protein with properties similar to those of a lectin. Two of the steps in the communication between bacterium and plant thus appear to have been outlined.

There has been a growing interest in the molecular biology of plant genes involved in nodulation and nitrogen fixation. Bisseling *et al.* (Agricultural University, Wageningen) and Verma *et al.* (McGill University, Montreal) reported the characterization of several nodule-specific, plant-encoded RNA species which code for nodule proteins (nodulins). Analysis of one of these components by Verma *et al.* showed that it was uricase, an enzyme important in the assimilation of fixed nitrogen. Ultrastructural analysis of nodules using labelled antibody to uricase showed that it was located not in the plant cells which contained bacteroids, but in adjacent uninfected cells. Using a different approach (subfractionating nodules) Schubert *et al.* (Monsanto Agricultural Products, St Louis) also identified a number of enzymes important in nitrogen assimilation in uninfected plant cells.

The identification of the locations of plant-encoded nodule enzymes is essential for an understanding of symbiotic nitrogen fixation. The location of the oxygen-binding protein leghaemoglobin has been a source of controversy although evidence has been accumulating that it is present within the plant cell cytoplasm rather than within the peribacteroid space (although the definitive experiments have yet to be done). As discussed by Robertson (DSIR, New Zealand) and Appleby (CSIRO, Canberra) the presence of leghaemoglobin in the cytoplasm suggests that it does more than supply oxygen to the bacteroids. Because the cytoplasmic concentration of free oxygen would be very low (in the μM range), the question arises as to whether the mitochondria (and other oxygenases) could operate satisfactorily at low oxygen tension. The future analysis of the plant's role in the symbiosis looks to be a promising area, especially now that Jacobsen (University of Groningen) has isolated mutant strains of peas which can be nodulated at levels of fixed nitrogen that would normally inhibit nodulation, and Larue *et al.* (Cornell University) have isolated a nodulation deficient mutant of pea.

Many other areas of the conference were of great interest. For example Haaker (Agricultural University, Wageningen) questioned the generally accepted mechanism of electron transfer within the nitrogenase enzyme complex; Leigh (University of Sussex) questioned if dinitrogen was bound end-on or side-on at the active site of nitrogenase; and Witty *et al.* (Rothamstead Experimental Station) questioned the validity of the use of the acetylene reduction assay which is usually used to assess the levels of nitrogen fixation occurring in plants. □

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Allan Downie is in the Department of Genetics, John Innes Institute, Colney Lane, Norwich, NR4 7UH.

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