but rapid growth that enables them to become so large.

Why are some giant clams brightly coloured? We don't know. The mantles of some giant clam species are plain brown, reflecting the colour of the zooxanthellae within. Most giant clams have coloured and patterned mantles, with some being spectacularly brilliant (Figure 1D). The adaptive significance of these patterns and spectacular colours has yet to be determined.

Are giant clams endangered? Giant clam species are extinct or in danger of extinction in many parts of their distributions, despite being listed in the IUCN Red List of Threatened Species and Appendix II of the Convention on International Trade in Endangered Species (CITES). The shells of the smaller species are often found in shell and tourist shops around the world. The large species have been fished commercially around the Indo-Pacific region at appalling levels by 'clam boats'. The market is for their adductor muscle, the large muscle that closes and holds the shells together. It weighs about 900 g in large clams and between 100–400 t of this muscle meat were landed in Taiwan each year over a 9-year period. This corresponds to about 300,000 clams/year and a total harvest of millions over two decades of illegal fishing. We are custodians of the largest bivalved animal that has ever lived and the Great Barrier Reef is the only region where its survival is assured.

Where can I find out more?

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Primer

Legume nodulation

J. Allan Downie

For reasons that are unclear, no eukaryotic enzymes can break the triple bond of N_2. The reduction of N_2 to NH_3 (nitrogen fixation) is limited to prokaryotes and is catalysed by nitrogenase. Since most of the nitrogen entering the biosphere (around 100 million metric tonnes of N_2 per annum) does so through nitrogenase activity (lightning contributes about 10%), those plants that associate with nitrogen-fixing bacteria have a significant selective advantage under conditions of limiting nitrogen.

Legumes optimise this advantage by entering into a symbiosis with rhizobia, a diverse group of bacteria that have inherited, by horizontal gene transfer, the ability to infect legume roots and form nitrogen-fixing nodules. These nodules are specialised 2–5 mm diameter organs that are usually formed on roots. Each nodule can contain up to 10^9 rhizobia in a niche perfectly suited for N_2 reduction by providing a supply of carbon (as malate) to the bacteria and a low O_2 environment. Non-legumes (*e.g.* alder, casuarina) in families phylogenetically related to the legumes can also form nitrogen-fixing nodules, but these nodules are occupied by filamentous nitrogen-fixing bacteria in the genus *Frankia*.

The relative ease of rhizobial and legume genetics coupled with biochemistry and cell biology have given considerable insights into this symbiosis. The analysis of the signalling system that initiates nodule morphogenesis and the development of root infection strategies in legumes has also helped in understanding both the nitrogen-fixing *Frankia* symbioses and arbusclar mycorrhizal symbioses that promote uptake of nutrients such as phosphate and nitrogen.

Nodules: a host-controlled niche for nitrogen fixation

During the intracellular infection of nodule cells, rhizobia differentiate into 'bacteroids' and are surrounded by a membrane of plant origin. The resulting structures are called symbiosomes, and in many respects act as plant organelles. However, instead of reducing only O_2 or CO_2 as done by mitochondria or chloroplasts, they reduce N_2 and so can be thought of as 'ammoniaplasts'.

Rhizobial nitrogenase is a complex made up of six protein subunits (two each of NifH, NifD and NifK) and contains two [4Fe–4S] and two (Fe_8S_7) iron–sulfur clusters and two iron–molybdenum cofactors (Fe_7MoS_9N) called FeMoco, which is the site of N_2 reduction. These nitrogenase metallo-centres are all very oxygen labile and must operate in an environment with a low level of free oxygen. Paradoxically, rapid respiration is required to produce the 16 ATP required for each N_2 reduced. Legume nodules resolve these paradoxical requirements of low free O_2 levels coupled with a rapid rate of O_2 transfer to energise nitrogen fixation, by expressing high levels of leghaemoglobins. These are O_2-buffering proteins similar to myoglobins that provide oxygen to mitochondria in muscle cells, but leghaemoglobins maintain much lower levels of O_2. The bacteroids carry out oxidative phosphorylation using a cytochrome oxidase (cytochrome cbb_3) with a high affinity for O_2. The net effect allows high levels of ATP synthesis with no oxidative damage to nitrogenase. In addition to intracellular O_2 buffering, O_2 diffusion into the nodule is regulated by an oxygen-diffusion barrier near the nodule periphery.

A successful symbiosis requires limitation of bacterial growth within nodules. Legumes control this growth in different ways. Firstly, although rhizobia produce NH_3, they cannot use it for growth, because they switch off their glutamine synthase, the primary enzyme for assimilation of NH_3. This is an astonishing development, because all of the NH_3 produced must go to the plant — the corollary is that rhizobia depend on nitrogen supplied by the plant. Work with rhizobial amino-acid transport-defective (*aap, bra*) mutants revealed that during their growth in pea nodules, rhizobia require branched-chain amino acids — possibly by supplying these, legumes 'persuaded' the bacteroids to rely on amino acids rather than NH_3 assimilation. A second level of control is that rhizobia lack the gene required for the synthesis of

homocitrate, and rely on the nodule-expressed plant gene product (FEN1) to synthesise this essential precursor for FeMoco synthesis from acetyl-CoA and α-ketoglutarate. Therefore, in principle, legumes can restrict where and when rhizobia make nitrogenase. Some legumes, including those in the Galegoid group of legumes (including peas, clover, alfalfa and other *Medicago* species) impose another level of control — they synthesise nodule-specific, cysteine-rich peptides (NCRs) that are translocated across the symbiosomal membrane and are targeted to the bacteroid membrane. These peptides cause bacterial endoreduplication without cell division and some permeabilisation of the bacteroid membrane. Although these enlarged bacteroids can efficiently fix nitrogen, they have lost the ability to regenerate and so pose no threat. The infecting bacteria still benefit, because some bacteria within nodule infection threads survive and can grow when the plant dies.

Legumes also limit the number of nodules formed, thereby balancing the overall rate of N-fixation with plant growth. The system controlling this is referred to as autoregulation of nodulation, and depends on a signal produced during the formation of nodule primordia. This signal is translocated to shoots where it is thought to induce the production of a signal that is then translocated to roots to suppress the number of nodules formed. Mutations in a leucine-rich repeat receptor kinase (called SUNN, HAR1 or NARK in *Medicago truncatula, Lotus japonicus* and *Glycine max*, respectively) with similarity to CLAVATA1 in *Arabidopsis* cause high levels of nodulation; grafting mutant shoots onto wild-type roots (and vice versa) showed that control by SUNN, HAR1 and NARK is exercised primarily by the shoots and not the roots. CLAVATA1 in *Arabidopsis* binds to CLE3, a short peptide and this binding regulates meristem formation. CLE-like peptides are induced in response to rhizobia and some of these can suppress nodulation and so are candidates for a signal from root to shoot; however, the root- and shoot-produced signals that control nodule number have not yet been identified. Running in parallel with this auroregulation is the fact that nodulation is repressed when soil nitrogen is abundant, and this can still occur in the autoregulatory mutants.

Legumes supply nutrients such as malate, SO_4^{2-}, Fe^{2+}, Mg^{2+}, and MoO_4^{2-} across the peribacteroid membrane and this is a potential level of control; the legume transporters for SO_4^{2-} and Fe^{2+} (Sst1 and Dmt1, respectively) have been characterised, but others have yet to be identified. In addition, legumes produce phytoalexins and antimicrobial metabolites such as canavanine that can limit bacterial growth during infection. Clearly, legumes are in control of the symbiosis and the bacteria can only activate nodule morphogenesis, induce infection and fix nitrogen if the conditions are right for the plant.

Specificity in signalling for nodule development and infection

Establishing nitrogen-fixing nodules requires two developmental programmes, one leading to nodule morphogenesis and one to the formation of infection threads (the plant-made structures through which rhizobia grow to reach the developing nodule). Rhizobia are adept at attaching to legume root hairs, where they are well placed to deliver signals that activate these development programmes. There can be specificity in the attachment — in pea, for example, a root-hair-expressed lectin binds to a specific rhizobial surface glucomannan, resulting in bacterial attachment to growing root-hair tips, the ideal location from which to initiate infection.

Flavonoids, isoflavonoids and related compounds from legume roots bind to rhizobial transcriptional activators (NodD), thereby inducing transcription of nodulation (*nod*) genes. The different NodD proteins in different rhizobia are adapted to recognising diverse flavonoids, isoflavonoids and related compounds produced by different legumes. In rhizobia (with a few notable exceptions), the induced *nod* gene products make nodulation factors (Nod factors), which are N-acylated chitin oligomers. Their synthesis requires NodC, which synthesises chitin oligomers, NodB, which deacetylates the terminal (non-reducing) N-acetyl glucosamine on the oligomer, and NodA, which transfers an acyl group (C_{16}–C_{22}) to the resulting free amino group. Various other *nod* gene products decorate these Nod factors with acetyl, methyl, carbamoyl, sulfuryl and glycosyl groups. Specificity in signalling is conferred by combinations of these decorations along with variations in the chain length (four or five N-acetyl glucosamine residues) and attachment of N-acyl chains of different lengths and degree of unsaturation. The Nod factors are recognised by membrane receptor-like kinases, thereby inducing root-hair deformation, activating nodule development and initiating the first steps of infection (Figure 1). Specificity in Nod-factor binding is critical for recognition between the prospective symbiotic partners (sometimes even distinguishing between varieties of a single legume species), adding to the layers of specificity related to root attachment and rhizobial *nod* gene induction.

In several legumes there is another layer of specificity determined by proteins secreted by rhizobial Type I, Type III, Type IV and Type VI secretion systems. Although their precise mechanisms of action are not fully understood, these secreted proteins normally complement Nod-factor-induced signalling. A few rhizobia can even induce nodulation without producing Nod factors — nodulation by one such strain of *Bradyrhizobium japonicum* is fully dependent on the Type III secretion system. The secreted proteins (or protein) responsible for this Nod-factor-independent nodulation have not yet been identified. Conversely, in some specific legumes, the intracellular injection of proteins by Type III and Type IV secretion systems appears to activate plant defence systems, causing inhibition of nodulation.

Signalling pathway activating nodule development

Analysis of loss- and gain-of-function nodulation mutations, particularly in the model legumes *M. truncatula* and *L. japonicus*, has identified components in the signalling pathway that leads to nodule development (Figure 1). Nod-factor binding to a receptor complex at the plasma membrane leads to activation of perinuclear calcium spiking, which activates a kinase that activates transcription of nodule development genes.

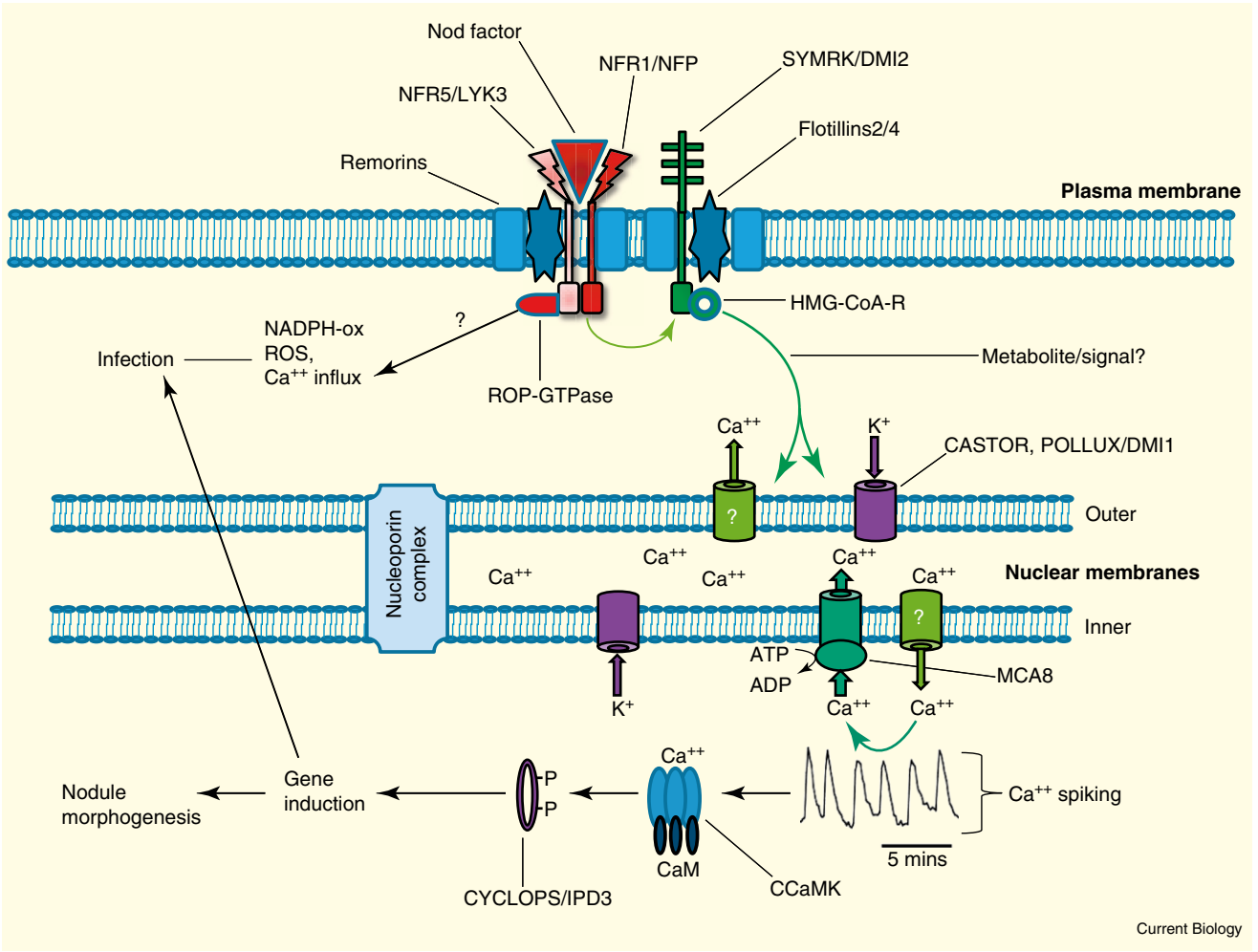

Figure 1. Model of Nod-factor-induced nodulation and infection signalling in legumes.
Nod factors bind to the LysM (lysin motif) domain serine/threonine receptor-like kinases NFR1/NFP and NFR5/LYK3, which probably form a lipid-raft-type complex on the plasma membrane, interacting with symbiosis-specific remorins and flotillins. Nod-factor recognition activates two outputs, one leading to nodulation morphogenesis and one proposed to be an additional response that is involved in infection. In the nodule signalling pathway the leucine-rich-receptor kinase SYMRK/DMI2 interacts with 3-hydroxy-3-methylglutaryl-CoA-reductase which is presumed here to make a secondary messenger. This unknown messenger is presumed to activate calcium spiking, possibly by opening of potassium-selective channels (CASTOR and POLLUX/DMI1) and/or an unknown calcium channel. Ca^{++} from the lumen between the nuclear membranes flows into the nucleoplasm and cytoplasm and is pumped back by an ATP-dependent pump MCA8. This together with repeated closure and opening of the K^+ and Ca^{++} channels could result in calcium oscillations, which activate the calcium and calmodulin-binding kinase CCaMK that is nuclear located. This kinase phosphorylates the transcriptional activator CYCLOPS which in turn induces the expression of transcriptional regulators. The resulting gene induction leads to nodule morphogenesis. Rhizobial infection requires this signalling pathway, but also requires an additional output from Nod-factor binding. It is proposed that this could be activated via a ROP-GTPase that binds to NFR5; ROP-GTPases are known to be involved in induction of NADPH oxidases, resulting in the production of reactive oxygen species and a calcium influx across the plasma membrane of growing root hairs. Since Nod factors induce ROS and a Ca^{++} influx and an NADPH oxidase is required for infection, it is speculated that this together with the genes induced by activated CYCLOPS/IPD3 may contribute to initiation of infection. (The alternative names of *L. japonicus*/ *M. truncatula* proteins are shown in that order.)

Nod factors bind to the extracellular LysM (lysin motif), chitin-binding domains of plasma-membrane serine/threonine receptor-like kinases. In *L japonicus*, mutations affecting the Nod-factor receptors NFR1 and NFR5 block root hair deformation and almost all responses associated with initiation of the symbiosis and the same is true of NFP, the *M. truncatula* orthologue of NFR5. The *M. truncatula* receptor most similar to NFR1 is LYK3 and although mutations in LYK3 block nodulation, some early signalling responses are retained, possibly because of genetic redundancy. NFR1 and NFR5 interact and bind Nod factors, activating kinase activity. A symbiotic-specific remorin (SYMREM1) and two flotillin-like proteins (FLOT2 and FLOT4) are thought to act as membrane chaperones forming a signalling complex in a lipid raft during infection thread growth and similar such signalling complexes may be functional in root hairs.

Also located on the plasma membrane is a leucine-rich repeat receptor (LRR) kinase (known as SYMRK in *L. japonicus* or DMI2 in *M. truncatula*) that must act downstream of the Nod-factor receptors, because the mutants retain some responses such as root-hair deformation. The anticipated ligand that binds to the extracellular LRR domain has

not been identified and so the role of SYMRK is not fully understood. The kinase domain of SYMRK/DMI2 interacts with a 3-hydroxy-3-methylglutaryl-CoA reductase, an enzyme normally involved in mevalonate biosynthesis and so it is thought that mevalonate or a related metabolite could be a secondary signal that transmits perception of Nod factors to the nucleus (Figure 1).

This putative secondary signal is thought to activate calcium oscillations both within and around the periphery of the nucleus. The oscillations are driven by a store of calcium between the inner and outer nuclear membranes, a compartment that is contiguous with the endoplasmic reticulum. Potassium-selective channels (CASTOR and POLLUX in *L. japonicus* and DMI1 in *M. truncatula*) in the nuclear membrane are required for calcium spiking. A calcium ATPase (MCA8) on the nuclear membrane is required to pump calcium back into the store and it is anticipated that a calcium channel will also be required to allow the rapid efflux of calcium (Figure 1). How the oscillations are activated has not yet been established, but it is thought that secondary signal(s) could activate the calcium/potassium channels to allow ion fluxes — the role of the potassium channels could either be to activate the calcium channel by changing the membrane potential and/or to allow potassium ion movement to charge-balance the rapid calcium efflux (Figure 1). Also required for calcium spiking are three symbiosis-specific nucleoporin components, NUP85, NUP133 and NENA. The role for nucleoporins has not been established; one possibility is that they may play a role in targeting components of the signalling pathway to the nuclear membrane.

The calcium oscillations within the nucleus activate a calcium and calmodulin-dependent kinase (CCaMK) unique to plants. The kinase contains three EF hands for calcium binding and a calmodulin-binding domain. CCaMK gain-of-function mutations induce spontaneous nodule morphogenesis in the absence of Nod factors, showing that this protein is sufficient to activate nodule development. Several of these are missense mutations inducing spontaneous nodulation by preventing phosphorylation of a threonine residue, by changing it to residues that cannot be phosphorylated. Structural modelling suggests that the phosphorylated threonine hydrogen bonds with an arginine on another loop of CCaMK — mutation of the arginine residue also causes spontaneous nodulation. Since deletion of the calmodulin-binding domains also induces spontaneous nodulation, the current model is that CaM binding in response to calcium spiking allows activation of the CCaMK kinase by inducing a conformational change that prevents formation of hydrogen bonding between the phospho-threonine and the arginine residues.

CCaMK phosphorylates a transcription factor called CYCLOPS (Figure 1), which is required for the expression of NIN, a transcriptional regulator required for nodule initiation and infection thread development. The regulation of the *NIN* promoter appears to be complex, because also required for *NIN* expression are NSP1 and NSP2, two GRAS-domain transcription factors; NSP1 induces expression of the transcription factors NIN and ERN1, and NSP2 binds to NSP1. Possibly there is a synergistic action between CYCLOPS and NSP1/NSP2. CYCLOPS is phosphorylated at two serines by CCaMK and substitution of both serine residues with aspartate (a phosphomimic) can give rise to an autoactive form of CYCLOPS that induces spontaneous nodulation; this requires both NSP1 and NSP2.

Once induced by nodulation signalling, NIN binds to the promoters of *NF-YA1* and *NF-YB1*, encoding two components of a heterotrimeric nuclear factor complex that activates the cell cycle. The resulting gene induction is probably important for both initiation of infection in root hairs, and activation in cortical cells of the cell cycle and cell divisions required to form a nodule meristem. Nodule morphogenesis requires a signal to be transmitted from the epidermis (where Nod-factors are perceived) to cortical cell layers where cell division is initiated. How this is achieved is not known but it is clear that cytokinin plays a role, because a gain-of-function mutation in the *L. japonicus* cytokinin receptor LHK1 causes spontaneous nodule formation and a similar effect is seen in *M. truncatula*. Loss of LHK1 function initially blocks nodule morphogenesis but infection threads proliferate in the cortex, showing that LHK1 is not required for infection.

Auxin is associated with nodule development and there is localised accumulation of auxin and accumulation of the auxin influx transporter AUX1 in response to rhizobia and Nod factors. These auxin changes may be caused by local inhibition of polar auxin transporters by accumulation of flavonoids. Auxin is required for lateral root development and a parallel role for auxin in nodule development appears likely. Even in spontaneous nodulation gain-of-function mutants, the nodules formed are discrete, indicating that nodule morphogenesis is not simply due to general activation of cell division, but probably requires gradients of plant hormones. Nodules usually initiate opposite protoxylem poles, and the hormone ethylene seems to suppress nodule development at other locations in the root.

The ethylene-insensitive *M. truncatula* mutant *skl* (equivalent to ethylene insensitive mutant *ein2* in *Arabidopsis*) produces increased numbers of nodules, the locations of which are less well determined relative to the protoxylem poles. A similar effect was seen when an ethylene-insensitive dominant mutant form of the ethylene receptor was expressed in legume roots. However, ethylene plays a complex role in this process. For example, ethylene represses Nod-factor-induced signalling in root hairs, and the *skl* mutant has enhanced nodulation, showing that ethylene can have an inhibitory role. However, ethylene-insensitive mutants in soybean and *L. japonicus* have reduced nodulation, and ethylene stimulates infection of the legume *Sesbania rostrata* under flooding conditions. Some of these differences may be related to specific roles of ethylene in some legumes, or different sites of ethylene action.

Nodule infection

Root infection is normally coordinated with nodule morphogenesis and usually involves the development of infection threads (ITs), plant-made tube-like structures formed by invagination of the plant cell membrane and the biosynthesis of plant cell wall along the growing

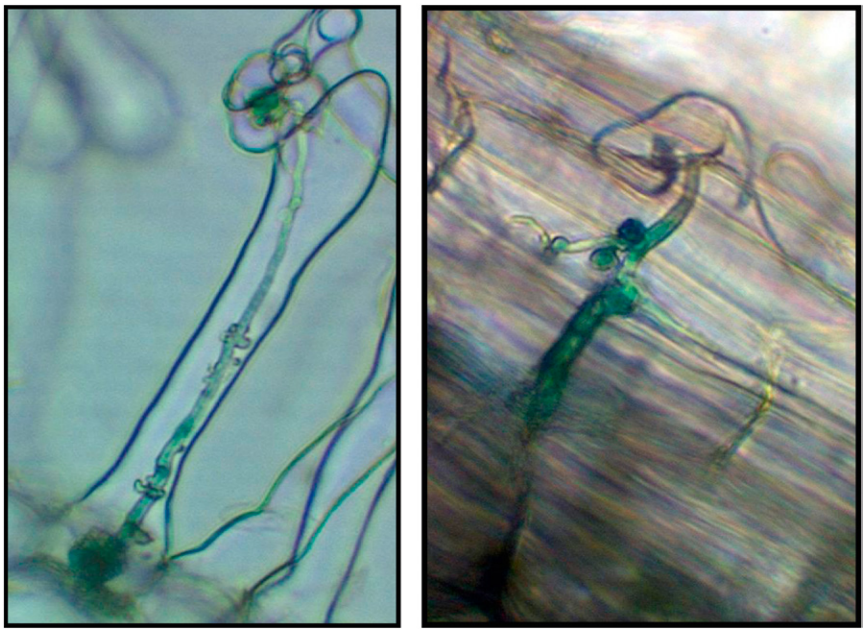

Figure 2. Infection threads induced in pea.
The image on the left shows an infection thread in a pea root hair that has curled back on itself. The bacteria within the infection thread expressed *lacZ* from a constitutive promoter and were stained using the chromogenic substrate X-gal. The image on the right shows another infection thread that has extended beyond the root hair cell into sub epidermal layers and small infection-thread branches can be observed. Images © Simon Walker, Babraham Institute, Cambridge.

invagination (Figure 2). The bacteria grow along the developing ITs, which grow between and through plant cells, eventually branching and ramifying through the developing nodule. Typically ITs initiate from root hairs that curl back on themselves, entrapping rhizobia in the crook of the curl, but ITs can also initiate from sites of intercellular infections at cracks in the epidermis (*e.g.* openings caused by emerging lateral roots). Accumulation of bacteria at specific loci seems to be critical for generating a signalling centre in which Nod factors accumulate.

Nod-factor addition can initiate formation of pre-infection threads (called PITs), observed as cytoplasmic bridges that define the path of IT growth. This involves cytoskeletal rearrangements that appear to be coordinated by the nucleus. It is thought that the plant cell cycle is activated and DNA replication occurs, but then the cell cycle is arrested prior to entry into M phase, and nuclear and cell division do not occur. It has been proposed that a phragmosome is formed, but rather than forming a cell plate (as would be organised by phragmosomes following nuclear division), a tube is formed as a consequence of nuclear movement away from the site of infection initiation.

The initiation and growth of ITs requires the Nod-factor signalling pathway described above (Figure 1), but, at least in some legumes, is more stringent with regard to Nod-factor structures. Activation of CCaMK is essential, but not sufficient for infection thread growth, because ITs were not formed by rhizobia in Nod-factor receptor mutants which carry a gain-of-function form of CCaMK. In *L. japonicus*, the nodulation signalling genes *NFR1*, *NFR5*, *CCaMK*, *CYCLOPS*, *NSP1* and *NSP2* are required throughout nodule development and infection, whereas epidermal-specific expression of the nucleoporins and the ion channels CASTOR and POLLUX appear to be sufficient to allow nodule infection.

In addition to activating the calcium-spiking signalling pathway, there must be different outputs resulting from Nod-factor recognition by the Nod-factor receptors. Although Nod-factor-induced root-hair deformation is abolished by mutations in Nod-factor receptors, such deformation is retained in *ccamk* mutants and in mutants blocked for calcium spiking. Therefore, activation of Nod-factor receptors (even by Nod-factor concentrations as low as 10^{-12} M) can induce root hair deformation through a pathway independent of calcium spiking. At higher concentrations (10^{-9} M and above), Nod factors induce other responses, including the production of reactive oxygen species (ROS) and partial depolarisation of the root-hair plasma membrane due to a calcium influx followed by K^+ and Cl^- movements at the root-hair tip (Figure 1). These responses also require Nod-factor receptors but none of the other components of the calcium-spiking pathway. It has been proposed that as bacteria accumulate in infection foci, the local concentrations of Nod factors increase, thereby potentiating additional responses from Nod-factor receptors.

It makes sense for plants to impose strong selectivity during infection, because entry by pathogens or non-symbionts could be costly. The requirement of Nod factors for induction of the calcium influx (and by implication, membrane depolarisation and ROS production) is higher than for induction of calcium spiking, both in terms of Nod-factor concentration and structural specificity of the Nod factors. For example, the loss of an O-linked acetyl group on *S. meliloti* Nod factors has no effect on induction of calcium spiking, but increases, by two orders of magnitude, the concentration required to induce the calcium influx. It is not clear how different outputs can be generated from Nod-factor receptors. One possibility is that it may occur as a result of recruitment of additional Nod-factor receptors. Alternatively, it is possible it could be by recruitment of another signalling process and significantly in this regard, the intracellular domain of the Nod-factor receptor NFR5 can bind a ROP-GTPase (Figure 1). During root-hair growth, ROP-GTPases can bind to and activate NADPH oxidase, resulting in the production of ROS, which are associated with a calcium influx. Given that an NADPH oxidase is known to be required for infection, activation of ROP-GTPases could be an additional signal that may activate initiation of infection thread development, complementing signalling through calcium spiking, especially since infection thread

growth can be considered to be analogous to root hair growth, although directed backwards into the root hair.

Infection thread development requires actin-induced cytoskeletal changes. Nod factors decrease the presence of longitudinal thick actin bundles, causing accumulation of finer and more diffuse actin at the root-hair tips. This will require the polymerisation of G-actin through the action of a complex that is activated by the SCAR/WAVE complex. Mutations affecting NAP1, PIR1 and ARPC1, three of the components of the SCAR/WAVE complex, block most infections by rhizobia, demonstrating a requirement for actin in infection thread initiation.

In addition to synthesising the new cell wall of the infection thread, there must be localised degradation of the existing plant cell wall at the site of infection thread initiation to enable the rhizobia to enter the growing infection thread. A Nod-factor-induced nodulation pectate lyase (NPL) is required for rhizobial infection and there is evidence that other plant cell-wall degradation enzymes are induced by Nod factors. These enzymes must be targeted to precise sites at which the infection thread is initiated and/or where it crosses the plant cell wall. The process of cytoskeletal rearrangement, localised plant cell wall degradation and new synthesis of infection thread wall material must occur in the adjacent cell, as the infection thread crosses the plant cell wall to extend through that cell.

Several other genes are required for infection. Some of these appear to be regulatory, either at the level of transcription or at the level of protein sorting (*e.g.*, *RPG*, *FLOT2*, *FLOT4*, *SYMREM1* and possibly *VAPYRIN*) or protein turnover. CERBERUS/LIN is an E3 ubiquitin ligase-like protein required for infection. Conversely, PUB1 is an E3 ubiquitin ligase that is activated by LYK3, and decreased expression of *PUB1* can enhance infection. The precise roles of several of these components are yet to be established. The growth of infection threads will require localised cell wall and cell membrane synthesis. There will be associated insertion of membrane proteins and secretion of proteins and intermediates required for cell wall and membrane synthesis. All of this will require protein targeting, possibly analogous to that seen with the exocyst complex that holds vesicles ready for fusion with SNARE proteins that locally target proteins to the membrane.

There is also a role for an appropriate bacterial cell surface. Bacterial mutants with modified surface polysaccharides are defective for infection. It is thought that oligosaccharides derived from these polysaccharides may act as signals during infection, possibly suppressing plant defence, but no plant receptors have been identified.

Evolution of nitrogen-fixing nodules

It is now clear that nodulation signalling evolved as a modification of the much more ancient signalling pathway required for the establishment of arbuscular mycorrhizal symbioses. With the exception of the Nod-factor receptors, all of the nodulation signalling components leading to activation of calcium spiking and CCaMK are also required for this mycorrhizal symbiosis. There are close structural similarities between at least some of the mycorrhizal signals (Myc factors) and Nod factors. Therefore, it is to be expected that Myc-factor receptors will be similar, but a receptor specific only for Myc factors has not yet been identified, possibly due to genetic redundancy.

Arbuscular mycorrhization is thought to have been associated with the adaptation of plants to the terrestrial environment and there is fossil evidence for arbusculated roots in land plants that grew around 450 million years ago. This fits well with the observation that mycorrhization is seen in about 80% of land plants, and is present even in the most basal clades of plants. Arbuscules are intracellular tree-like branching structures that promote nutrient uptake to the plant (especially phosphate and nitrogen) in exchange for carbon, which can promote fungal growth outside of the root, giving the fungus greater access to nutrients, such as phosphate, which benefits the plant.

How bacteria acquired the ability to make Myc-like Nod factors is not known. One possibility is that some bacteria acquired the genes from mycorrhizal fungi. However, there is also the possibility that convergent evolution resulted in the biosynthesis of Nod factors — most bacterial cell walls contain peptidoglycan, which is made up of a backbone of β-(1,4) linked N-acetylglucosamine and N-acetylmuramic acid. Since N-acetylmuramic acid is a derivative of N-acetylglucosamine containing an ether-linked lactic acid, it would not be a large step to evolve the ability to make chitin oligomers (β-(1,4) linked N-acetylglucosamine). One step of Nod-factor synthesis is very unusual, namely the attachment by NodA of the fatty-acyl chain onto the deacetylated amino group of the terminal glucosamine of the preformed chitin oligomer. Perhaps identification of the origin of NodA will give some insight into where bacteria acquired Nod-factor biosynthetic capacity.

Presumably, some bacteria accrued benefits by acquiring the ability to make Myc factor-like signals. However, whether this bacterial acquisition occurred initially in the rhizobia is not clear, because parallel nitrogen-fixing symbioses developed with actinomycete bacteria in the genus *Frankia*. These bacteria form nitrogen-fixing nodules on species in the Cucurbitales, Fagales and Rosales orders, all of which are closely linked in phylogenetic terms to the Fabales order that contains the legumes. In some ways, the infection of roots by actimomycete bacteria such as *Frankia* spp. has more similarities to mycorrhizal infection because carbon acquired from the plant has the potential to directly benefit the extracellular bacteria by translocation through these filamentous bacteria.

It has been proposed that nodule-based nitrogen fixation evolved in two major steps, one providing a predisposition for nodulation and another that permits full development of nodules. This has been proposed because relatively few genera within the Cucurbitales, Fagales and Rosales produce nodules and so it is thought that nodule development evolved separately in only some lineages that have the predisposition to nodulation. In the Cucurbitales, Fagales and Rosales orders, fewer than 300 species have been identified as forming nitrogen-fixing nodules, and these species fall into about 25 of the 174 or so genera.

In marked contrast, around 16,000 species within the 650–700 genera of legumes have been identified as forming nitrogen-fixing symbioses with rhizobia. If such diversity is taken as a measure of evolutionary success or fitness, the legume family within the Fabales has been considerably more successful than any other family of nodulating plants in the closely-related orders. Why is it that nitrogen-fixing nodules on legumes should appear to confer so much benefit compared with the non-legume nodulating plants? One clear difference is the different bacteria that infect them. Possibly *Frankia* spp. are less efficient at fixing nitrogen than rhizobia. However, the wide diversity of rhizobia that can nodulate plants suggests that successful nitrogen-fixing symbioses can be established by very diverse bacteria, even between different divisions of the bacteria. It is not apparent why *Frankia* spp. did not evolve a symbiosis that was as mutually beneficial (based on relative evolutionary diversity) as that seen with rhizobial-legume symbioses.

The difference in the structures of legume nodules compared with non-legume nodules may give an insight into why legume nodules are so successful. Nodules on non-legumes are somewhat similar to short modified lateral roots with a central vasculature. In contrast, legume nodules have a peripheral vasculature. A clear advantage of a peripheral vasculature is that oxygen will be available for energy production by mitochondria associated with the vasculature, and so ATP generated by respiration would be readily available to drive the energy demands of the vasculature. This would not normally be a significant problem in roots, but in a nodule with a central vasculature, the haemoglobin in the cells surrounding the vasculature would tend to bind most of the available oxygen. I propose that a physiological limitation on nodules with a central vasculature could be the relative difficulty of respiration, and hence ATP synthesis in the cells of the vasculature. The identification of a gene (*COCHLEATA* in pea and *NOOT* in *M. truncatula*) that normally represses root identity in nodules may be the first step toward identifying how legumes differentiate a peripheral vasculature.

Other reasons for the relative efficiency of legume nodules could be related to the genome duplications that occurred in some of the legume families. These duplications could have enabled the acquisition of additional evolved functions in duplicated genes, to help develop the highly efficient nitrogen-fixing nodules in current legumes.

Further reading

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Rank influences human sex differences in dyadic cooperation

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Unrelated human males regularly interact in groups [1], which can include higher and lower ranked individuals. In contrast, from early childhood through adulthood, females often reduce group size in order to interact with only one individual of equal rank [1–5]. In many species, when either sex maintains a group structure, unrelated individuals must cooperate with those differing in rank [6]. Given that human males interact more than females in groups, we hypothesized that dyadic cooperation between individuals of differing rank should occur more frequently between human males than females. We examined this hypothesis in academic psychology. Numbers of co-authored peer-reviewed publications were used as an objective measure of cooperation, and professorial status as a measure of rank. We compiled all publications co-authored by full professors with same-sex departmental colleagues over four years in 50 North American universities, and calculated the likelihood of co-authorship in relation to the number of available professors in the same department (Supplemental information). Among those of equal status (full professors) there was no gender difference for likelihood of co-authorship: women and men were equally likely to co-author publications with another full professor of the same gender. In contrast, male full professors were more likely than female full professors to co-author publications with a same-gender assistant professor. This is consistent with a tendency for men to cooperate more than women with same-sex individuals of differing rank.

We first tabulated the mean numbers of female full professors (M = 5.28), male full professors (M = 9.50), female assistant professors (M = 3.84) and