

ticks correspond to the rounds of the cell-division cycle? And is something similar at work in other ageing neuroblasts, and in other animal species?

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## Plant biology

# Locks, keys and symbioses

Martin Parniske and J. Allan Downie

The association between legumes and nitrogen-fixing bacteria requires molecular recognition to allow bacterial entry into root hairs. The discovery of a novel type of plant receptor clarifies how this happens.

Legumes, such as pea, bean, trefoil and peanut, are agricultural wonders. They form symbioses with bacteria, known as rhizobia, which means that they can make their own nitrogen fertilizer by 'fixing' atmospheric nitrogen. The fixation process occurs in root nodules. But this is the end-point of a developmental programme, which starts with a molecular recognition system that enables root hairs to promote the entry of rhizobia while excluding a huge diversity of unwelcome intruders from the soil. In 1990, the structure of one component of the recognition system was published<sup>1</sup>. That component, which comes in various types, is the Nod factor. It is produced by rhizobia and is the key that they need to open the root-hair lock and enter the plant.

Since 1990, identification of the corresponding plant receptor for Nod factors has been a central goal of research. Papers by Radutoiu *et al.*<sup>2</sup> and Madsen *et al.*<sup>3</sup> (pages 585 and 637 of this issue), and a report in *Science* by Limpens *et al.*<sup>4</sup>, now describe a new class of receptor kinases that could constitute the lock for the Nod-factor key. Kinases are enzymes that add phosphate groups to other proteins: they act as molecular switches, which turn enzymes or signalling pathways on or off.

In legumes, the root hairs exhibit an astonishing developmental switch once molecular recognition occurs between rhizobia and a plant, and the entrance mechanism is triggered. The tip of a root hair stops growing in a straight line and the cell curls back on itself, trapping bacteria within a pocket. The bacteria grow in the pocket and are taken up into a plant-made intracellular 'infection thread' (Fig. 1). Overall, the root-hair response involves a complex redirection of cellular development, requiring orchestration by positional and chemical cues.

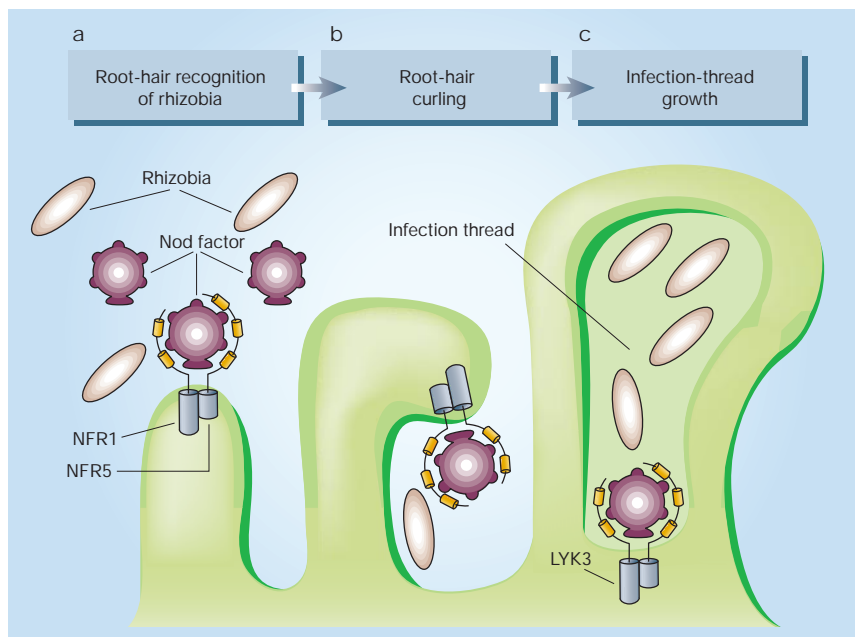
Radutoiu *et al.*<sup>2</sup> worked with mutants of the legume *Lotus japonicus*. They found that plants with mutations in two genes, *NFR1* or *NFR5*, do not respond to Nod factor and are not infected by rhizobia. Nod factors have a

backbone of several *N*-acetylglucosamine molecules, which are 'decorated' with various other chemical groups depending on the Nod factor concerned. Radutoiu *et al.* demonstrate that *NFR1* and *NFR5* proteins carry two or three extracellular LysM motifs. The significance of this is that the LysM motif occurs in a variety of enzymes that bind to polymers containing *N*-acetylglucosamine<sup>5,6</sup>. So it seems that the *NFR* proteins bind Nod factors through their extracellular LysM domains, and that binding elicits signalling via the intracellular kinase domain. The LysM receptors probably act in concert with

other players such as *SYMRK*<sup>7,8</sup>, another receptor kinase, but one that lacks LysM motifs in its extracellular domain.

Radutoiu *et al.* show that the earliest detectable root-hair responses to Nod factor, typically observed within minutes, are completely abolished in *nfr5* mutants and modified in *nfr1* mutants, but are retained in a *symrk* mutant. Given this, it looks as if *NFR5* and *NFR1* act sequentially and individually, followed by *SYMRK*. But it is also possible that different combinations of receptors form recognition complexes. This would resolve one puzzle — the *NFR5* kinase does not have an 'activation loop', a usual feature of these enzymes; therefore, because it is predicted to lack kinase activity, *NFR5* probably relies on an interaction with another protein to initiate signalling.

The *NFR1* and *NFR5* proteins are clearly required during the earliest stages of the rhizobium–plant interaction (Fig. 1a, b). But other results indicate that LysM receptors are also involved in later events. Rhizobia continue to produce Nod factor within infection threads, and Madsen *et al.*<sup>3</sup> show that the pea version of *NFR5*, *SYM10*, is expressed at a higher level in tissue that contains growing infection threads. But we cannot yet tell whether *NFR5/SYM10* is required for sustained infection-thread growth because of the block on thread initiation in *nfr5/sym10* mutants.



**Figure 1** Unlocking the root-hair door. a, b, Two receptors enable a plant to sense the Nod factor produced by the 'right' symbiotic rhizobia, and then allow root-hair curling for the rhizobia to enter. Work with *Lotus japonicus* shows that the receptors, *NFR1* and *NFR5*, act at the earliest stage in events<sup>2,3</sup>. c, The probable *Medicago truncatula* variant of *NFR1*, *LYK3*, appears to be required to maintain infection-thread growth<sup>4</sup>; the expression pattern<sup>3</sup> of the pea version of *NFR5*, *SYM10*, suggests that this protein might also be required at this stage. But it remains to be seen if *NFR1*, *NFR5* and/or *LYK3* interact as speculated here. Overall, it looks as if there could be a continuous requirement for *NFR1* and *NFR5*, or their counterparts in other legume species, throughout root-hair curling and infection-thread formation.

In their work, Limpens *et al.*<sup>4</sup> started with a specific line of pea that has an unusual pattern of Nod-factor recognition. The root hairs curl and entrap rhizobia. But the process stops after infection-thread initiation unless the rhizobia produce a Nod factor that has specific decorations<sup>9,10</sup>. Limpens *et al.* mapped the overall genetic locus, *SYM2*, responsible. In another species, *Medicago truncatula*, which has a smaller genome, they narrowed down the *SYM2* target region to a stretch of DNA about 300 kilobases in length. Surprisingly, it contained many excellent candidates for involvement in Nod-factor perception — among them seven receptors potentially involved in disease resistance, and seven receptor kinases with extracellular LysM domains. Each candidate gene was silenced using an RNA interference strategy. Only with one, called *LYK3*, did a symbiotic defect result: when *LYK3* expression was reduced, Limpens *et al.* found that although infection-thread growth was initiated, it subsequently ceased. This effect depended on the structure of the Nod factor produced by the bacteria.

The authors' interpretation is that there is a second lock-and-key mechanism in the developing infection thread, and that the second lock is encoded by *LYK3* (Fig. 1c). Because of similarity in sequence, and a similar genomic environment, this gene is likely to be the *Medicago* version of the *Lotus NFR1*. Curiously, however, *Medicago* plants with silenced *LYK3* and *Lotus nfr1* mutants differ substantially. One possible explanation stems from Limpens and colleagues' observation that silencing of *LYK3* in their system was incomplete. The residual *LYK3* expression could be sufficient to establish the first lock, but not the second. Once pea variants of *LYK3* have been identified, it will be possible to find out whether they contribute to the unusual pattern of Nod-factor recognition of the pea line that initially sparked the interest in the *SYM2* genetic locus.

There is also an evolutionary angle to the new findings. Chemical decoration of Nod factors varies depending on the rhizobial strain, and it determines the specificity of a host plant and its bacterial symbiont<sup>11</sup>. The genes encoding Nod-factor receptors appear to evolve rapidly, because very closely related plants can prefer different Nod-factor structures. *LYK3* (ref. 4) and *NFR1* (J. Stougaard, personal communication) reside in clusters of several similar genes, in tandem array, which may be the clue to how the corresponding sequence diversification is achieved. By facilitating recombination between diverged gene copies within the array, such an arrangement is suitable for generating new sequence variants, and new recognition specificities, relatively quickly<sup>12</sup>.

There is much to learn. In particular, it remains to be seen exactly how the different LysM kinases integrate into a signalling

pathway (or network) to guide root-hair curling, and initiate and sustain infection-thread growth. Another major task is to find out whether, or how, Nod factors and LysM receptor kinases physically interact. ■

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## Nuclear physics

# It's a knockout

David Warner

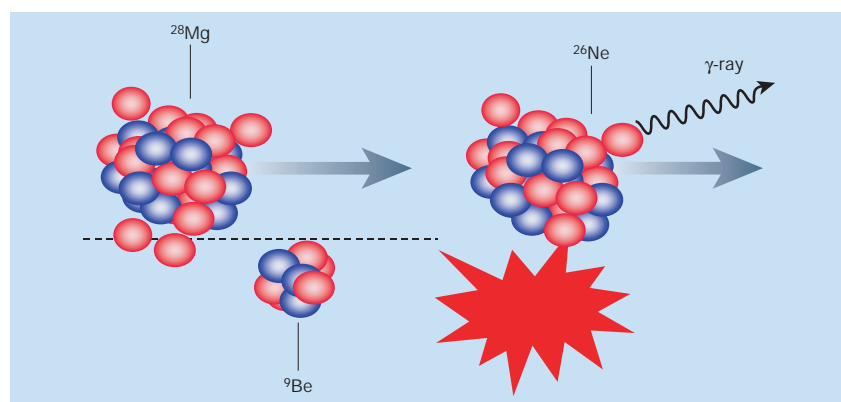
In collisions between nuclei, a proton or neutron might be knocked out of one nucleus. Now, two-proton knockout has been demonstrated, opening a new route to the creation of neutron-rich systems for study.

Most of our knowledge about the quantum nature of the atomic nucleus comes from the study of nuclear reactions in which an energetic beam of one nuclear species is directed onto a target made of another. Writing in *Physical Review Letters*, Bazin and colleagues<sup>1</sup> have now demonstrated that it is possible to knock two protons simultaneously out of a nucleus in the high-energy beam, while leaving the remaining nucleons (neutrons and protons) largely undisturbed. This observation of a so-called 'direct' reaction process offers a valuable tool to probe some of the most exotic regions of the nuclear chart.

When two nuclei react in a collision, there is a plethora of possible outcomes, because there are many different ways in which the interaction can take place. One class of reaction<sup>2</sup>, referred to as 'direct', has proved crucial in elucidating the motion of the individual nucleons inside a nucleus. In a high-energy

direct reaction, the projectile undergoes a peripheral, grazing collision with the target nucleus, and only the surfaces interact. This type of reaction occurs quickly, compared with, say, a 'compound' reaction in which the two nuclei collide violently and the energy is shared out among all of the constituents of the combined system. In the direct reaction, there is then a simple relationship between the physical descriptions, known as wavefunctions, of the incoming and outgoing systems; in a knockout reaction, for example, they differ only through the addition or removal of one or two nucleons. The reaction thus creates a new nucleus and simultaneously provides detailed information about the quantum states that its nucleons occupy.

Until recently, the chief constraint on experiments exploring the nuclear many-body system was that, in any reaction chosen for study, both beam and target had to be stable, naturally occurring species. But the



**Figure 1 Two-proton knockout.** Bazin *et al.*<sup>1</sup> have demonstrated that the incidence of a beam of unstable magnesium nuclei (<sup>26</sup>Mg) on a target of stable beryllium nuclei (<sup>9</sup>Be) produces a residue of neon (<sup>26</sup>Ne) nuclei. During the reaction, two of the four loosely bound, outermost protons in the beam nucleus are removed, leaving the <sup>26</sup>Ne nucleus, which then emits  $\gamma$ -radiation. The velocity of the residue is the same as that of the beam particles.