How CYCLOPS keeps an eye on plant symbiosis

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The availability of phosphorus and nitrogen are major limitations to plant growth, and as such our agricultural processes apply these nutrients at high concentrations to crop plants through fertilizer. Although fertilizer application has greatly enhanced food production it comes at a significant price: the chemical fixation of nitrogen depends on high levels of fossil fuels, making fertilizers a significant cost of food production and a major cause of greenhouse gas emissions from agriculture. A number of plants have entered beneficial interactions with microorganisms that facilitate the uptake of nitrogen and phosphorus from the soil. In this issue of PNAS Yano *et al.* (1) provide new insights into a novel genetic component in the plant that allows the establishment of these nutrient-capturing symbioses.

Plants enter a symbiosis with fungi of the taxonomic group Glomeromycota, which facilitates phosphate and mineral acquisition (2, 3). Fungal hyphae invade the plant root and form highly-branched intracellular intrusions into cortical cells, called arbuscules, where nutrient exchange occurs. A more limited set of plant species also forms interactions with rhizobial bacteria that facilitates nitrogen uptake. A novel organ, the nodule is formed on the roots of the host plants and bacteria are accommodated in plant membrane-bound compartments within the nodule cells where nitrogen fixation occurs (3). Legumes, peas and beans, are able to form both mycorrhizal and rhizobial symbioses. Genetic studies on nodulation in legumes revealed that several genes were required for both nodulation and mycorrhization, resulting in the identification of 7 distinct loci making up the so-called "common symbiosis signaling (Sym) pathway" (2, 4, 5). Thus far the Sym pathway consists of up to 2 putative cation channels (MtDMI1, LjCASTOR and LjPOLLUX), 1 leucine-rich repeat receptor-like kinase (MtDMI2, LjSymRK), 1 calcium- and calmodulin-dependent kinase (CCaMK), and 2 members of the nuclear pore complex (NUP85, NUP133). Today, we have reached a landmark in this pioneering age with the report by Yano *et al.* (1) of the cloning of the seventh gene in this pathway: *CYCLOPS*.

The Sym pathway is necessary for the recognition of the rhizobial signal Nod factor (6). Perception of Nod factor involves lysin motif-containing receptor-like kinases (LysM-RLK) that have a specific function in Nod factor recognition with no apparent role in the establishment of the mycorrhizal symbiosis (7–10). Downstream of these LysM-RLKs is the Sym pathway that is involved in the activation and perception of an oscillatory calcium signal: calcium spiking (reviewed in ref. 5). It is widely believed that CCaMK is responsible for decoding and transmitting the calcium signal. Specificity is possibly encoded in the frequency of the oscillation as calcium oscillations differ between Nod factor and mycorrhizal treatment (11). Downstream of CCaMK the pathway diverges with nodulation-specific transcription factors [NSP1, NSP2, and ERN1 (12–15)]. Analogous transcription factors in the mycorrhizal-specific branch have not yet been identified.

Mutations in *CYCLOPS* are less penetrant than mutants in other Sym pathway components.

Characterization of *cyclops* reveals that this gene functions at an equivalent position as CCaMK in the Sym pathway. *CYCLOPS* encodes a protein with a C-terminal coiled-coil motif and 2 nuclear localization signals that direct the protein to the nucleus, colocalizing with CCaMK. *CYCLOPS* is orthologous to the *Medicago truncatula* gene *IPD3* that was identified in an interaction screen with *M. truncatula* CCaMK (16). CYCLOPS interacts with CCaMK in vivo, requiring a functional kinase domain, and CYCLOPS is phosphorylated in vitro by CCaMK. From this work it is difficult to predict the exact function of CYCLOPS: it may act downstream of CCaMK transmitting the calcium signal to the transcription factors, or it may act as a modulator of CCaMK that is itself modified by CCaMK.

CYCLOPS differs from other members of the Sym pathway in that *cyclops* mutants show more significant responses to mycorrhizal fungi and rhizobial bacteria: *cyclops* can initiate bacterial infection and nodule primordia and show greater levels of mycorrhizal infection than mutants in other Sym pathway components. These findings indicate that mutations in *CYCLOPS* are less penetrant than mutants in other Sym pathway components, although *nup133* and *nup85* also show some initiation of nodule primordia. A valuable assay has emerged for testing the relevance of nodulation genes for nodule organogenesis: a point mutation in the autophosphorylation site of CCaMK leads to a gain-of-function that induces the formation of nodules in the absence of rhizobia (17, 18). When *cyclops* was transformed with this construct spontaneous nodules were observed. From this Yano *et al.* (1) conclude that unlike other members of the Sym pathway *CYCLOPS* is not required for nodule organogenesis. However, while the CCaMK gain-of-function induced spontaneous nodules in *cyclops*, the numbers of nodulating plants and the number of nodules per plant was greatly reduced compared with wild type, which can be interpreted as a function for *CYCLOPS* during nodule organogenesis.

Yano *et al.* (1) suggest that *CYCLOPS* represents a branch point in the pathway: rhizobial infection is *CYCLOPS*-dependent, whereas nodule organogenesis is *CYCLOPS*-independent. *CYCLOPS* would then belong to an ancient pathway required for infection of fungi that was recruited later on for the infection of rhizobia (19). In this rationale, bacterial infection threads may have evolved from the prepenetration apparatus present in plant cells preparing for mycorrhizal invasion (19). An additional program encoding nodule organogenesis was then superimposed on this ancestral pathway and requires a pathway downstream of *CCaMK* but independent of *CYCLOPS* (Fig. 1*A*). However, this is an abrupt break from the current model as *CYCLOPS* would be the only Sym pathway component of seven not required for nodule primordium initiation. Also, it would imply a dual role for the downstream transcription factors *NSP1*, *NSP2*, and *ERN1*, all of

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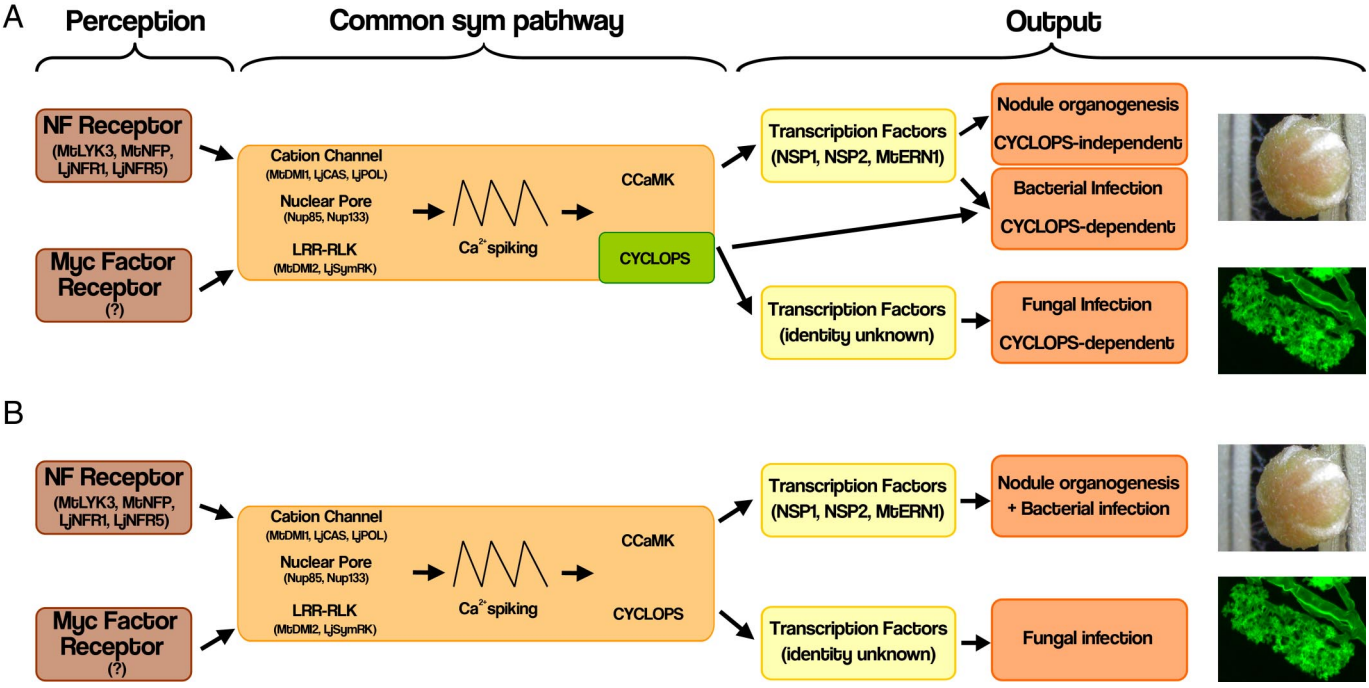

Fig. 1. Two alternative models for the role of *CYCLOPS* in symbiosis signaling. (*A*) The model of the Sym pathway according to Yano *et al.* (1). Symbiotic signals are perceived by appropriate receptor complexes, activation of which will lead to a signaling cascade within the Sym pathway. Because *cyclops* mutants show spontaneous nodulation it is interpreted that *CYCLOPS* acts as a branching point in the pathway, where fungal and bacterial infection are regulated in a *CYCLOPS*-dependent manner and nodule organogenesis is regulated in a *CYCLOPS*-independent manner. This finding suggests that *NSP1*, *NSP2*, and *ERN1* transcription factors have dual roles in both the bacterial infection branch and the nodule organogenesis branch as mutants in these genes are impaired in both. (*B*) An alternative model for the Sym pathway. Because *cyclops* mutants show greatly reduced levels of spontaneous nodulation it is interpreted that *CYCLOPS* is required for nodule organogenesis, but that the *cyclops* mutants have low penetrance. Low penetrance allows a more parsimonious Sym pathway in which *CYCLOPS* has analogous functions to all other members of the Sym pathway. The pathway bifurcates into nodulation-specific and mycorrhization-specific branches, each governed by their own set of transcription factors. NF, Nod factor. Pictures of a root nodule on *L. japonicus* and an arbuscule of *Glomus versiforme* in an *M. truncatula* root cortical cell stained with WGA Alexa Fluor 488 are shown at right.

which are involved in both nodule organogenesis and bacterial infection (Fig. 1*A*). A second interpretation of the spontaneous nodulation data are that the poor penetrance of *cyclops* mutants allows low levels of spontaneous nodulation, but that *CYCLOPS* is required for nodule organogenesis. The second model provides a much simpler model for the Sym pathway, where all components of the pathway are fulfilling equivalent functions in laying the developmental frameworks associated with bacterial infection, nodule organogenesis, and mycorrhizal invasion (Fig. 1*B*).

This last decade has seen significant advances in the genetic dissection of the Sym pathway in legumes. The cloning of *CYCLOPS* represents the end of an era, being the final member of the Sym pathway to be defined in this early genetics work. This genetics has provided us with the anatomy of the Sym pathway. Our challenge now is to take this genetics into a coherent understanding of the mechanisms of symbiosis signaling.

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