

# To be or *noot* to be

## Evolutionary tinkering for symbiotic organ identity

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**Abbreviations:** AM, arbuscular mycorrhization; BOP, blade-on-petiole; COCH, cochleata; LCO, lipochitooligosaccharide; MYA, million years ago; MycLCOs, mycorrhization factors; NF, Nod factors; NFC, nitrogen fixing clade; NOOT, nodule root; RNS, root nodule symbiosis

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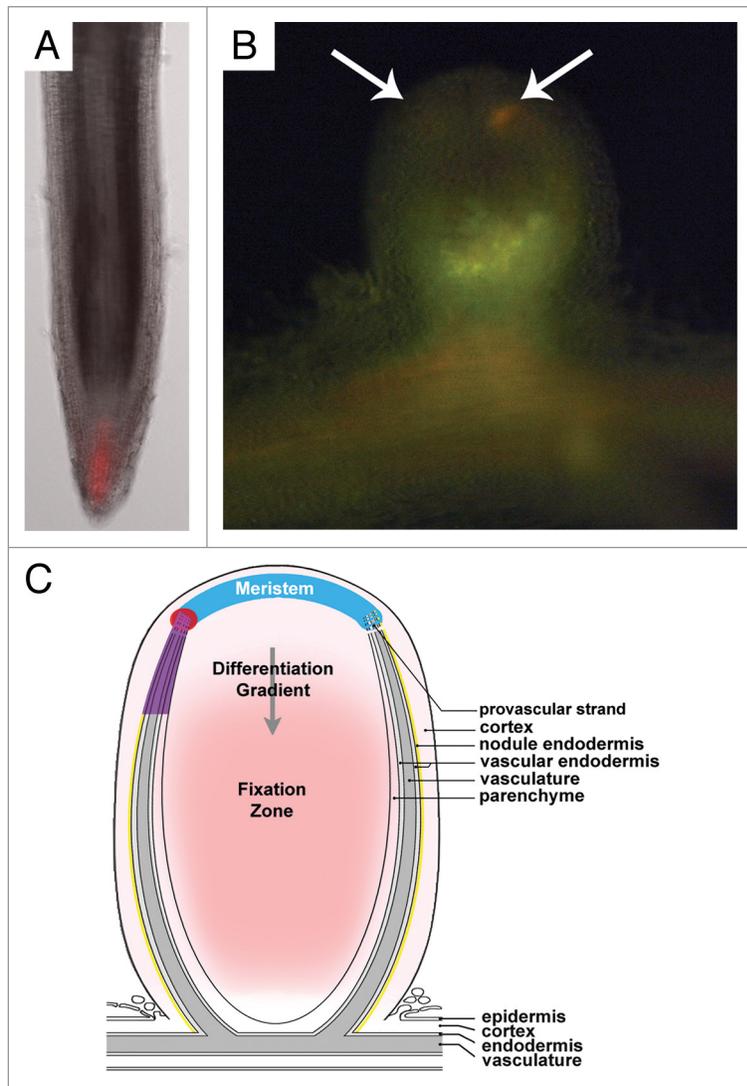
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Legume plants develop symbiosis specific organs on their roots as a result of their interaction with rhizobia. These organs, called nodules, house the nitrogen fixing bacteria. The molecular mechanisms governing the identity and maintenance of this organ are still poorly understood, but it is supposed that root and nodule development share common features. We have identified the *Medicago truncatula nodule root (NOOT)* and *Pisum sativum cochleata (COCH)* orthologous genes as necessary for the robust maintenance of nodule identity throughout the nodule developmental program. *NOOT* and *COCH* are *Arabidopsis blade-on-petiole (BOP)* orthologs and *NOOT* and *COCH* show functions in leaf and flower development in *M. truncatula* and *P. sativum* respectively that are conserved with the functions of BOP in *Arabidopsis*. The characterization of the *noot* and *coch* mutants highlights the root evolutionary origin of nodule vascular strands and suggests that the *NOOT* and *COCH* genes were recruited to repress root identity in the legume symbiotic organ.

The capacity to establish an endosymbiosis with soil bacteria called rhizobia is predominantly found in plants from the legume family (Fabaceae). This interaction leads to the de novo formation of symbiotic organs called nodules, generally formed on roots of the plant hosts. This symbiotic association allows the plant to overcome nitrogen limitation by taking advantage of bacterial nitrogenase activity. In return, the plant provides carbon derivatives to its symbionts.

Root nodule symbiosis (RNS) has been acquired by co-option of (part of) the mycorrhizal symbiotic machinery as exemplified by the presence of a signaling pathway in plants that is common to both symbioses and constituted of at least seven genes.<sup>1-5</sup> The arbuscular mycorrhizal (AM) symbiosis is widespread in land plants,<sup>6</sup> from liverworts to Angiosperms,<sup>7</sup> and results from the association between the root of a host plant and soil fungi from the *Glomeromyceta* clade. The acquisition of mycorrhizal ability in plants occurred at least 400 million years ago (MYA) and is thought to be one of the crucial events that has contributed to the successful land colonization by the green lineage.<sup>8,9</sup> The aptitude to develop the RNS appeared more recently (around 100 MYA) and is restricted to a subclade of Eurosid I plants, including the Fabacea.<sup>10,11</sup> This subclade was named the nitrogen fixing clade (NFC).<sup>10</sup> The genetic pre-disposition of the NFC and the key molecular events that have allowed acquisition of the RNS have not been clearly established. Identifying this(ese) factor(s) could lead to the possibility transferring this unique symbiotic property to non-nodulating plants.<sup>12</sup> At a fundamental level, unravelling these factors represents a particularly interesting question in order to understand the acquisition of both new morphogenetic capacities and new plant-microbe signaling pathways.

In agreement with the common origin of plant symbiotic signaling pathways, the structure of microbial symbiotic signals, mycorrhization factors (Myc-LCOs) and nod factors (NFs), are similar. Both



**Figure 1.** Auxin accumulates at the ends of nodule vascular strands. Expression of the *revDR5:mRFP* construct in *M. truncatula* roots (A) and nodules at 10 d post inoculation (B). (A) Observation under bright field and fluorescence light. (B) under fluorescent light. The construct is expressed predominantly in the root columella (A) and at the tip of the vascular tissue in nodules (indicated by the arrows in (B)). The scheme in (C) indicates where the *revDR5:mRFP* (red spot) and the *promNOOT:reporter* construct (purple area) are expressed in the nodule (they are only shown on one side to allow labeling of nodule tissue).

have a lipo-chitoooligo saccharidic (LCO) backbone.<sup>13</sup> Remarkably, LCOs stimulate lateral root formation,<sup>13,14</sup> suggesting that prior to triggering rhizobial infection and nodule organogenesis, LCO factors were used as root morphogens to facilitate symbiotic interactions. In contrast to nodule formation that appears to be cytokinin-dependent,<sup>3,15</sup> lateral root initiation requires auxin signaling. In addition, the two organs have distinct histological organizations with a central vasculature in roots in contrast to peripheral vascular strands in many legume nodules.<sup>16</sup>

However, the nodules formed in actinorhizal plants and *Parasponia* have a central vasculature derived from the pericycle with peripheral infected tissues that suggests a root derived origin and could represent a more ancestral state of the legume symbiotic organ.<sup>17</sup>

The symbiotic *noot* and *coch* mutants develop roots in apical positions of their symbiotic organs.<sup>18,19</sup> These homeotic mutants represent interesting genetic tools to understand the identity and the origin of the symbiotic organ. The formation of ectopic roots from indeterminate and

determinate nodules (i.e., with or without a persistent apical meristem, respectively) was previously reported as a result of increased temperature in *Medicago sativa* and *Trifolium repens* species,<sup>20,21</sup> nodulation of composite plants in *Arachis*<sup>22,23</sup> or after inoculation with rhizobium mutant strains in *Phaseolus*, *Trifolium*, soybean and mungbean plants.<sup>24-27</sup> In two of these studies, the ectopic roots and the nodule vasculature were unambiguously connected.<sup>22,25</sup> The existence of these *noot*-like phenotypes in determinate nodules shows that the conversion of a nodule into a root can be independent of the presence of a persistent meristem.

Our work showed that the *NOOT* and *COCH* genes are necessary for the robust persistence of the nodule. *NOOT* and *COCH* are *Arabidopsis* BOP orthologs and their functions in the definition of lateral aerial organs are conserved in *M. truncatula* and *P. sativum*.<sup>19,28,29</sup> This suggests that *NOOT* and *COCH* were recruited from aerial developmental programs to repress root identity in the legume symbiotic organ. Alternatively, *NOOT* BOP *COCH* LIKE proteins may fulfill a discrete, and as yet uncharacterized, function during root development. In the *noot* and *coch* mutants, the ectopic roots do not derive from the nodule meristem but originate from the vascular initials<sup>19</sup> as suggested in the other systems.<sup>22,25</sup> Furthermore, the *WOX5* root identity marker is expressed in the nodule vasculature extremities.<sup>30</sup> This suggests that, in contrast to the central part of the symbiotic organ that is developing in response to cytokinin,<sup>3,15</sup> the nodule vascular strands are ontologically related to roots.

As root formation is an auxin dependent process, we investigated auxin distribution in nodules. To do this, we used the auxin responsive *revDR5:mRFP* construct.<sup>31</sup> As expected, when this construct was present in *Medicago* composite plants, fluorescence was similar to that described in *Arabidopsis* root tips, from the quiescent center cells to the columella<sup>31</sup> (Fig. 1A). In nodules, a signal was observed at the ends of vascular strands toward the nodule apex (Fig. 1B) indicating auxin accumulation and a root meristem-like identity.

In *Arabidopsis*, the BOP proteins are expressed at aerial tissue boundaries and

appear to act within the leaf to control development at the shoot apical meristem.<sup>32</sup> Similarly to its *Arabidopsis* ortholog the *NOOT* expression pattern<sup>19</sup> suggests a function in the delimitation of boundaries, in this case between the nodule meristem and the vascular tissue initials in the symbiotic organ. As for the shoot and root meristems, and in agreement with the enlarged meristematic regions in the *noot* and *coch* mutants,<sup>19</sup> nodule organogenesis may require fine-tuned distribution and responses to both cytokinin and auxin to coordinate meristem activity and vascular tissue development. We thus propose that *NOOT/COCH* may participate in this regulation by defining the boundaries between the meristem and vascular tissue territories. In conclusion, the *noot* and *coch* mutants reveal that during evolution, legume plants have recruited pre-existing developmental elements to create an apparently unrelated organ,<sup>33</sup> the symbiotic nodule.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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