An SHR–SCR module specifies legume cortical cell fate to enable nodulation

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Legumes, unlike other plants, have the ability to establish symbiosis with nitrogen-fixing rhizobia. It has been theorized that a unique property of legume root cortical cells enabled the initial establishment of rhizobial symbiosis[1–3]. Here we show that a SHORTROOT–SCARECROW (SHR–SCR) stem cell program in cortical cells of the legume *Medicago truncatula* specifies their distinct fate. Regulatory elements drive the cortical expression of *SCR*, and stele-expressed SHR protein accumulates in cortical cells of *M. truncatula* but not *Arabidopsis thaliana*. The cortical SHR–SCR network is conserved across legume species, responds to rhizobial signals, and initiates legume-specific cortical cell division for de novo nodule organogenesis and accommodation of rhizobia. Ectopic activation of SHR and SCR in legumes is sufficient to induce root cortical cell division. Our work suggests that acquisition of the cortical SHR–SCR module enabled cell division coupled to rhizobial infection in legumes. We propose that this event was central to the evolution of rhizobial endosymbiosis.

The anatomy of the nodule in legume plants was described in the 17th century, and nodule cells were found to host endosymbiotic rhizobia for nitrogen fixation in the 19th century[4–6]. The legume cortex is developmentally distinct from the cortex of non-legumes: it can de-differentiate in response to phytohormones or symbiotic signals from rhizobia, thereby enabling de novo organogenesis of nodules to accommodate nitrogen-fixing rhizobia[1,7–13]. Nevertheless, why symbiotic nitrogen fixation is restricted to relatively few plant species, mainly in legumes, has remained unclear.

SCARECROW is expressed in legume cortical cells

To identify genetic pathway reprogramming events that might underlie the cortical cell division response in legumes, we generated EGFP–β-glucuronidase reporters (*EGFP-GUS* driven by the promoter for the gene of interest) for *M. truncatula* and *A. thaliana* genes whose homologues have essential developmental roles in non-legumes, and identified those that were differentially expressed in *M. truncatula* hairy roots (Extended Data Fig. 1).

SCR is an important stem cell regulator in plant roots[14]. Reporter expression driven by the promoter for *A. thaliana SCR* (*pAtSCR:EGFP-GUS*) was limited to the quiescent centre and endodermis in both *M. truncatula* hairy roots and stably transformed *A. thaliana* (Fig. 1a, Extended Data Fig. 2a), as expected[9,14]. By contrast, the reporter for *M. truncatula* (*pMtSCR:EGFP-GUS*) was expressed not only in the endodermis, but also in the cortex and to a lesser degree in the epidermis of *M. truncatula* transformed hairy roots (Fig. 1a, Extended Data Figs. 1, 2b), as confirmed by in situ hybridization (Extended Data Fig. 2c).

To identify regulatory elements in the *MtSCR* promoter that are required for cortical expression, we examined a series of *MtSCR* promoter deletion reporters in *A. thaliana* and identified two *cis*-regulatory elements: an AT1-box[15,16] and an enhancer[17]. The AT1-box was first identified within promoters of certain photoregulated genes and binds a nuclear protein in pea[15], whereas the enhancer was identified within the photoregulated and CO_2-responsive *Cah1* promoter in the green alga *Chlamydomonas reinhardtii*[17]. Individual deletion of the AT1-box (−1,604 base pairs (bp) to −1,615 bp; *ΔAT1*) and the enhancer (−1,632 bp to −1,638 bp; *ΔEn*) led to a reduction in reporter gene expression in cortical cells in *A. thaliana*, and deletion of both elements (*ΔEnΔAT1*) largely abolished reporter gene expression in the cortex (Extended Data Fig. 2a). The *MtSCR* reporter lacking both elements was weakly expressed in *M. truncatula* hairy root cortical cells (Fig. 1a).

The AT1-box and enhancer were typically found within 100 bp of each other in the *SCR* promoters from legumes (Extended Data Fig. 2d). By contrast, these elements were further from each other, or one was absent, in the *SCR* promoters in species outside the nitrogen fixation clade (Extended Data Fig. 2d). Notably, other legume plants, including *Glycine max*, *Lotus japonicus*, *Cicer arietinum*, *Pisum sativum* and *Lupinus albus*, also expressed their *SCR* orthologues in cortical cells (Extended Data Fig. 2e, f). Together, these data suggest that expanded *SCR* expression is conserved in legumes and requires the AT1-box and enhancer elements within the *SCR* promoter, but their proper spacing, higher-order structure, or other components may regulate *SCR* promoter activation in the cortex.

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