A switch in Ca^{2+} spiking signature is concomitant with endosymbiotic microbe entry into cortical root cells of *Medicago truncatula*

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During the initial stages of root colonization by either nitrogen-fixing rhizobia or arbuscular mycorrhizal (AM) fungi, the microsymbionts traverse outer root tissues within specialized intracellular compartments of plant origin, thus avoiding direct contact with the host cytoplasm and the activation of defence responses. In order to study the mechanisms underlying this unique form of transcellular infection we have developed *in vivo* experimental approaches based on confocal microscopy for the model legume *Medicago truncatula* with the objective of monitoring host cellular dynamics and associated intracellular signaling. This original approach has revealed a key role for the host nucleus in orchestrating endosymbiotic infection, and in particular that polarised nuclear migration directs the construction and orientation of the growing intracellular compartments. Furthermore, we know from previous studies that an essential host signaling response to the presence of symbiotic microbes involves the activation of repeated oscillations of intranuclear calcium ions known as Ca^{2+} spiking.

In this article we have addressed the role of microbe-plant communication during the complex transcellular infection process by monitoring host Ca^{2+} spiking throughout colonization of the root outer cortex of *M. truncatula*.

Live-tissue imaging performed with FRET-based calcium sensors known as cameleons has revealed that Ca^{2+} spiking is indeed activated in individual cells during specific stages of endosymbiotic infection. Strikingly, we observe a rapid switch from low to high frequency spiking during the earliest stages of host cell entry for both bacterial and fungal symbionts. These findings imply that Ca^{2+}-mediated microbe-plant signaling plays an important role in reprogramming host cell development specifically related to controlled endosymbiotic infection.

Host Ca^{2+} signaling during rhizobial infection

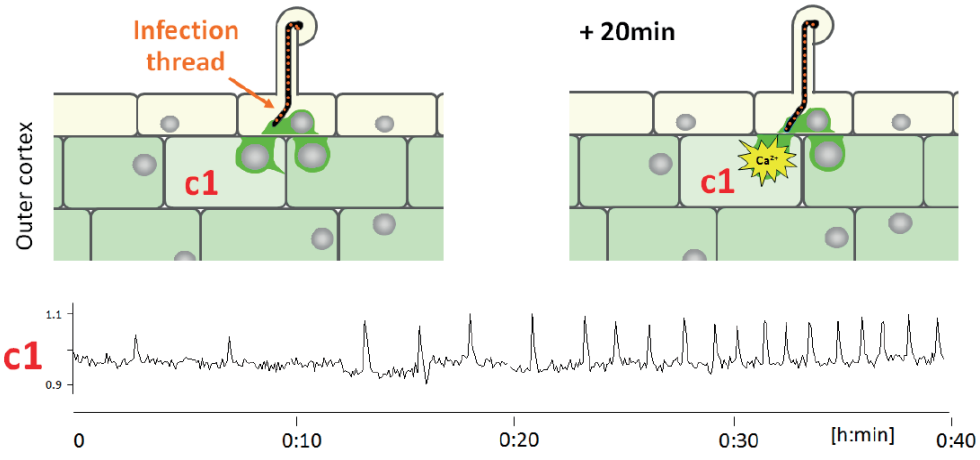

Figure Legend

High frequency nuclear Ca^{2+} spiking accompanies initial rhizobial colonization of the *M. truncatula* root outer cortex. The left-hand image shows the nuclear positioning and cytoplasmic remodeling which occurs within the outer cortical cell c1 prior to infection.

The *Rhizobium*-containing infection thread (IT) has just reached the base of the epidermal root hair, and at this stage there is only rare Ca^{2+} spiking in the underlying cell c1. Approximately 20 min later (right-hand image) the IT is about to enter the cortical cell and this is accompanied by a rapid increase in spiking frequency shown in the profile below. This highly characteristic host cell response signals a critical stage of commitment to symbiotic microbial infection and associated cell developmental re-programming.

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