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Article Addendum

Calcium opens the dialogue between plants and arbuscular mycorrhizal fungi

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Calcium ion is considered a ubiquitous second messenger in all eukaryotic cells. Analysis of intracellular Ca^{2+} concentration dynamics has demonstrated its signalling role in plant cells in response to a wide array of environmental cues. The implication of Ca^{2+} in the early steps of the arbuscular mycorrhizal symbiosis has been frequently claimed, mainly by analogy with what firmly demonstrated in the rhizobium-legume symbiosis. We recently documented transient Ca^{2+} changes in plant cells challenged with diffusible molecules released by arbuscular mycorrhizal fungi. Ca^{2+} measurements by the recombinant aequorin method provided new insights into the molecular communications between plants and these beneficial fungi.

In the rhizosphere plants meet a wide array of microorganisms. In favorable interactions, such as arbuscular mycorrhizal (AM) and nitrogen fixing symbioses, a dialogue is progressively established between the two interacting organisms to make the appropriate partner choice. These two-way communications rely on the interchange of signals released by both potential symbionts. After perception of the signalling molecules, a signal transduction pathway is induced, leading to the activation of the proper genetic and developmental program in both partners.

Variations in intracellular free Ca^{2+} concentration occur as one of the initial steps in signalling pathways activated in plants when they encounter pathogens,[1] fungal biocontrol agents[2] and nitrogen-fixing bacteria.[3] Molecules secreted by microorganisms, after binding to specific receptors, trigger in plant cells transient changes in cytosolic Ca^{2+} level, due to the influx of the ion from the extracellular environment and/or the release from internal Ca^{2+} storage compartments.[4,5] Ca^{2+} messages delivered to plant cells are at least partly deciphered on the basis of their spatial and temporal features. The occurrence of different Ca^{2+} signatures guarantees the specificity of the ensuing physiological responses.

In the legume-rhizobium symbiosis a definite pattern of Ca^{2+} oscillations has been reported to occur in response to the rhizobial signalling molecule, the Nod factor, in the nucleus and perinuclear cytoplasm of the root hair.[6] The Ca^{2+} spike number has been recently demonstrated to regulate nodulation gene expression.[7]

Legumes are able to engage in a dual symbiotic interaction, with rhizobia and AM fungi. Components of the Ca^{2+}-mediated signalling pathway are shared by the two symbioses.[8] In the mycorrhizal signal transduction pathway the involvement of Ca^{2+} has long been speculated, based on the observed similarities with symbiotic nitrogen fixation.[3]

To evaluate the possible participation of Ca^{2+} in the early steps of the AM symbiosis, we have used a simplified experimental system given by plant cell suspension cultures stably expressing the bioluminescent Ca^{2+}-sensitive reporter aequorin.[9] The use of cultured cells circumvents the problem posed by multilayered organs: in aequorin-transformed seedlings, possible Ca^{2+} changes occurring in rhizodermal cells—the first place where the AM fungal signals are perceived and transduced—can be misrecorded due to luminescence calibration over all root cell layers, resulting in an underestimation of the Ca^{2+} signal in the responsive cells. An experimental design based on challenging host plant cells with the culture medium of different AM fungi (*Gigaspora margarita*, *Glomus mosseae* and *intraradices*) provided the first firm evidence that Ca^{2+} is involved as intracellular messenger during mycorrhizal signalling, at least in a pre-contact stage. Cytosolic Ca^{2+} changes, characterized by specific kinetic parameters, were triggered by diffusates obtained from AM resting and germinating spores,[9] and extraradical mycelium.[10] Cultured plant cells demonstrated to be competent to perceive the diffusible signal released by AM fungi and to decode the message in a Ca^{2+}-dependent pathway. Based on these experiments, it seems that AM fungi announce their presence to the plant through the constitutive release of a chemical signal, even before experiencing the proximity of the plant or its AM symbiotic signals. The notion that the secreted fungal molecules herald, through Ca^{2+}, a beneficial message which can be acknowledged only by competent receivers, is supported by: (1) the lack of defense response induction and the upregulation of some genes essential for the AM symbiosis initiation in host plant cells; (2) the unresponsiveness of cultured cells from the nonhost plant *Arabidopsis thaliana*.

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Ca^{2+}-mediated perception of both AM fungal and rhizobial signals by plant cells unifies the signalling pathways activated in the two symbioses. However, the actual occurrence of Ca^{2+} spiking in AM symbiosis remains to be ascertained, due to limitations of the recombinant aequorin method, when applied to an asynchronous cell population. Contribution of internal Ca^{2+} stores, in particular the nucleus, to the observed Ca^{2+} changes will be a future research goal to be achieved through a pharmacological approach and/or targeting of Ca^{2+} indicators to intracellular compartments.

The identification of the plant-derived mycorrhizal signal as strigolactones[11] and their inducing activity on AM fungi[12] have represented a major breakthrough in the AM symbiosis research field. Elucidation of the chemical nature of the AM fungal factor, which plays several effects on host plants,[9,13-15] is eagerly awaited.

Understanding how AM fungi and rhizobia select compatible plant hosts, thus activating the appropriate symbiotic program, is another facet to be considered in the future to get a complete overview of early signaling events in legume symbioses. Analysis of Ca^{2+} signalling implication in the microbial partner would require the delivery of reliable and sensitive Ca^{2+} probes (such as aequorin- or GFP-based[16]) for Ca^{2+} measurements in living microorganisms. The recombinant aequorin method has been successfully applied to monitor dynamic changes in intracellular Ca^{2+} levels in the bacteria Anabaena sp.,[17] *E. coli*,[18] and recently by us in rhizobial strains.[19] Unfortunately, AM fungi have proved not to be amenable to stable transformation, being coenocytic, multinucleate and heterokaryotic,[20,21] and only transient transformants have been obtained so far.[22,23] Further development of the transformation technologies may provide in the future a valuable tool to analyse, from the fungal side, signal perception and transduction during arbuscular mycorrhiza establishment.

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