The symbiotic role of the actin filament cytoskeleton

Title page

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Legumes interact with soil-borne bacteria (rhizobia), thus developing a symbiotic association that allows atmospheric nitrogen fixation inside specialized plant organs called root nodules (Gage, 2004). Root nodule colonization requires the remodeling of plant cells, which develop a novel intracellular compartment where symbionts can be hosted: the symbiotic interface. This remodeling occurs in all colonized cells, starting from the epidermal root hairs, where bacteria enter the so-called infection thread (Fournier et al., 2008; 2015), a membrane-bound tube that channels rhizobia through the root cortex, into nodule primordia and within the internal tissues of mature nodules. While several studies have investigated plant cell responses during rhizobia uptake in outer root tissues, the study of bacterial release in the deep nodule tissues is far more challenging. A new paper by Zhang et al., 2018, present in this issue of New Phytologist (pag. XXX-XXX), now provides unprecedented insight in the organization of the actin microfilament cytoskeleton during this process.

Actin cytoskeleton can be described as the most fragile and dynamic component of the plant cell: imaging its structure in cells that lay deep into a plant organ is the nightmare of any plant cell biologist. In fact, live imaging of fluorescent signals from internal nodule tissues is simply not possible with existing equipment. The only option is, as was done by Zhang and colleagues, to section the nodule and image the intact cells in the layers that lay just beneath the cut. One can expect that this is not artifact free and the physiological state of the cells has to be checked constantly during imaging time. Without this, the risk of imaging cells that are severely stressed or even dead is substantial. Moreover, earlier reports show that the observed cytoskeleton depends on the marker and each marker has its characteristic labeling pattern (Wilsen et al., 2006, Melak et al., 2017, Montes-Rodriguez and Kost, 2017). Finally, the imaging of the actin cytoskeleton fine structure is out of the reach of a spinning disk confocal microscope and requires novel microscope techniques that permit higher resolution.

Zhang and colleagues made a first step towards live cell imaging of the actin filament cytoskeleton deep inside a plant organ with a mixed strategy, including fresh sectioning, chemical fixation, and the use of both exogenous (phalloidin) and endogenous actin markers (a GFP fusion with the second actin-binding domain of *Arabidopsis* fimbrin1). This combined approach provides us for the first time with a view of actin filament structure and dynamics in the inner tissues of root nodules.

The observations by Zhang and coworkers point - not surprisingly - at a dramatic reorganization of actin microfilaments throughout the process of nodule cell colonization (Figure 1). Infection threads are surrounded by a dense network of actin bundles, which also embrace infection droplets, the sites where membrane-bound rhizobia are released in the

cytoplasm, on their way to become N-fixing organelle-like bacteroids. In later stages of cell colonization, the number of bacteroids increases until they occupy most of the cell volume, surrounding the centrally-positioned vacuole in radially-oriented arrays. At this stage, actin reorganization reaches its maximum, with a diffuse network extending from the perivacuolar space into the mass of bacteroids. A remarkable feature that is described here for the first time is the appearance of an extensive number of very short - or dot-like - actin filaments. At first sight such patterns could be interpreted as initial stages of damage to cytoskeleton integrity. Nevertheless, after comparing fresh and fixed samples, the Authors conclude that such actin fragments are indeed a feature of cytoskeleton rearrangement during nodule cell maturation. Earlier studies on the actin cytoskeleton in pea (Davidson and Newcomb, 2001), soybean (Whitehead et al., 1998) and *M. truncatula* (Gavrin et al., 2015) were limited to chemically fixed nodules and in these studies the presence of actin filament fragments was either not noticed or attributed to an artifact of fixation. The question arises then if these structures are limited to nodules or do they constitute a general feature of the plant actin cytoskeleton that escaped attention so far?

These novel results complement with a previous study (Kitaeva et al. 2016), that investigated microtubule organization in nodule cells with comparably advanced approaches. By overlapping the two sets of information, we can now draw a more thorough model about the role of cytoskeleton in nodule cell differentiation and bacterial release. Microtubule bundles running along infection threads and wrapping around infection droplets overall mirror the present description of the actin filament cytoskeleton, suggesting that the two major components of plant cytoskeleton act in synergy during early cell colonization by the invading rhizobia. A partial parallelism can be drawn with actin and tubulin patterns along growing root hairs, where roughly longitudinal bundles of both components are observed along the hair shaft (Cárdenas et al., 1998; Sieberer et al., 2005): the common interpretation points at a cooperative action of microfilaments and microtubules fostering fast cytoplasmic streams that deliver secretory vesicles to the growing root tip. This similarity between root hair and infection thread-associated cytoskeletal patterns is in line with the vision of infection thread development as an inward-directed tip growth (Gage, 2004).

By contrast, an important difference marks tubulin and actin cytoskeleton in mature nodule cells. Microtubules have been shown to organize in radiating bundles that run along the arrays of bacteroids that surround the vacuole. This is only partially conserved in actin microfilaments, which organize in a more complex network: radial and more massive bundles

in fact associate with a blurred mesh of medium to very short filaments interweaving the mass of bacteroids.

Albeit further investigations will be required to clarify the respective roles of actin- and tubulin-based cytoskeleton in N-fixing cells, it is tempting to speculate that microtubules and large microfilament bundles act in positioning the bacteroid arrays and performing long-distance transport of interface-directed materials, whereas fine actin filaments might be involved in the maintenance of the peribacteroid membrane, the specialized interface mediating all nutritional exchanges between the symbionts. In particular, short actin microfilaments are often associated to the most dynamic plant cell membranes, with a primary role in vesicle delivery and membrane rearrangements, for example during cell plate formation (Verma, 2001) or tip growth (Hepler et al., 2001). Furthermore, a diffuse network of short actin bundles has been proposed to be acting in the feeding sites of root parasitic nematodes, made of multinucleated cells with intense secretory activity (De Almeida Engler et al., 2004). It is therefore not surprising to find this type of actin filaments in nodule cells that host hundreds of active membrane-bound bacteroids. To better investigate the role of each cytoskeletal component, it would now be interesting to use mutants in microtubule- or microfilament-associated motor proteins and record bacteroid positioning and functionality.

Comparing cytoskeletal organization upon rhizobial and arbuscular mycorrhizal fungal colonization (Genre and Bonfante, 1998) highlights an interesting analogy: fine meshes of short actin filaments embrace both bacteroids and arbuscule branches (even if dot-like patterns have not been described in the latter case). This similarity appears significant in the frame of our current understanding of arbuscular mycorrhizas and symbiotic nitrogen fixation: the two symbioses share several traits, starting from a common signaling pathway, and symbiotic nitrogen fixation is in fact believed to evolutionarily derive from the reprogramming of cell mechanisms already set in place by the more ancient arbuscular mycorrhizas (Parniske, 2008).

Figure legends

Figure 1. Schematic representation of actin organization and remodeling associated with rhizobium release and symbiosome development during symbiotic nodulation in *Medicago truncatula*. The actin cytoskeleton tightly encloses infection threads and infection droplets, guiding the elongation of infection threads and the rhizobial release (a). During later

steps of nodule cell colonization, a network of actin microfilaments embraces the developing symbiosomes (b), while in mature, nitrogen-fixing cells, a more complex network is described, radially aligned with mature symbiosomes around the central vacuoles, with the appearance of short actin fragments (c). The figure was kindly provided by Zhaosheng Kong.

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