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## The symbiotic role of the actin filament cytoskeleton

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1 **Title page**

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11

12 **Title**

13 **The symbiotic role of actin filament cytoskeleton**

14

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

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

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

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

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

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

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

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

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

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

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109

## 110 **Figure legends**

111

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

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

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