Title of Project

Actin visualization: to disclose mechanisms of host cell reorganisation during interactions with microbes

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Team

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Summary

The actin cytoskeleton is required for a multitude of plant cellular functions, including growth, development, cell architecture and response to microorganisms. Actin dynamics are involved in plant immunity and symbiotic interactions, to facilitate dramatic reorganization of the plant cytoskeleton. The aim of our project is to develop a multilevel resource for actin visualization and imaging in living cells of *Medicago truncatula* based on a genetically encoded fluorescent actin reporter. Medicago is the major model plant to study root nodule symbiosis and arbuscular mycorrhiza symbiosis as well as interactions with different leaf and root pathogens. However, actin labelling in Medicago is to date only achieved through root organ transformation. The establishment of a stable line with actin labelled in every cell including above ground tissue will be useful to a wide scientific community involved in plant-microbe interaction research as well as other research interests. As a powerful instrument it will enable new approaches and experiments, which are currently too complex for implementation. It will enable addressing new scientific questions and open problems and ultimately help to our better understanding underlying mechanisms of plant cell architecture rearrangement during interactions with leaf and root colonising microbes. Therefore, this resource will be exceptionally valuable for the development of new strategies of disease resistance breeding in crops.

Proposal

Actin filaments are a fundamental structural component of eukaryotic cells. Actin plays an important role in numerous aspects of plant cell growth and development, including responses to abiotic and biotic stimuli. Plants encounter and must deal with a range of other organisms that may be potential symbionts or pathogens. The plant cytoskeleton is instrumental in mediating the plant's response to it. Changes in the organization of the plant cytoskeleton during plant interactions with microbial and other organisms are complex and varied, and much still remains to be elucidated [1].

The advances in understanding the role of the actin cytoskeleton in plant development have come in large part from the improved ability to document its dynamics and organization in living cells. Actin imaging in living plant cells was achieved by genetically encoded filamentous actin reporters designed based on fluorescent protein fusions to actin binding domains of actin regulatory proteins. These are powerful tools to decipher the role of the actin cytoskeleton in plant growth and development. This progress was achieved in large part because of generation of stable transgenic lines of *Arabidopsis thaliana* and *Nicotiana tabacum* expressing fluorescent actin reporters [2].

However, progress in the investigation of the actin functionality in plant interactions with symbionts is hampered by the separation of plant model systems. Arabidopsis had been the most valuable model plant to work on genetics and various aspects of plant biology, but it does not establish interactions with the most valuable plant symbionts: root mycorrhiza fungi and nitrogen fixing rhizobia. *Medicago truncatula* has been used extensively for symbiosis research and has been instrumental for identifying genes affecting interactions with beneficial fungi and nitrogen-fixing rhizobia during root nodule symbiosis development. Moreover, Medicago is now an established model to study plant-pathogen interactions, seed and leaf development. The main disadvantage of Medicago as a model plant is time consuming genetics. Generation of a stable transgenic lines can take at least several months and significant efforts. That is why postdocs, who usually limited by two year contract terms, involved in actin projects about symbiosis development basically do not have time to generate an expressing fluorescent actin reporter line of Medicago. Instead, researchers have to use alternative techniques of actin visualization: immunolabelling, chemical staining, methods of transient transformation of plant organs, which all have their benefits and issues and often disregarded by reviewers (Fig. 1).

The **aim** of our project is to develop a multilevel resource for actin visualization and imaging in living cells of *Medicago truncatula* including:

1. The PhytoBricks standard parts containing the fluorescent actin reporter GFP-ABD2-GFP and a symbiotic specific Leghemoglobin (pLB) promoter.
2. Ready-to-use destination vector encoding a transcriptional module including two transcriptional units pUBQ:GFP-ABD2-GFP and pLB:GFP-ABD2-GFP.
3. Stable transgenic *Meicago truncatula* line expressing fluorescent actin reporter pUBQ:GFP-ABD2-GFP - pLB:GFP-ABD2-GFP.

Methods

The actin reporter based on the fimbrin Actin Binding Domain 2 (ABD2) flanked by green fluorescent proteins (GFP-ABD2-GFP) proved to be one of the best among other actin fluorescent reporters (as GFP-Talin or GFP-MBD). It has minimal side effects on plant growth and shows high quality actin labelling. It was also shown that the reported driven by Ubiquitin (pUBQ) promoter provides better visualization of actin dynamics in plant cells [2, 3]. However, the ubiquitin promoter is not active in symbiotic cells of Medicago nodules colonised by nitrogen-fixing rhizobia. To overcome this we will create a double-transcript construct. First, using the Golden Gate assembly methods we will generate PhytoBricks containing GFP-ABD2-GFP reporter and symbiotic specific Leghemoglobin promoter. Standard parts with Ubiquitin promoter and terminators are available at Addgene. Base on that we will create a destination vector encoding transcriptional module pUBQ:GFP-ABD2-GFP - pLB:GFP-ABD2-GFP (Fig. 1). Where pUBQ:GFP-ABD2-GFP is transcribed constitutively

and pLB:GFP-ABD2-GFP is active only in symbiotic cells of Medicago nodules. Therefore, it will enable actin imaging in all types of cells and tissues. This part of the project will be performed at SLCU Cambridge.

Then, we will introduce our vector into Medicago by means of *Agrobacterium*-mediated transformation followed by somatic embryogenesis regeneration resulting in stable transgenic Meicago line expressing fluorescent actin reporter. This part will be performed in collaboration with the Crop Transformation Group of Prof. Wendy Harwood, who run a platform for genome editing and genetic modification of various plants at The John Innes Centre, Norwich.

Benefits and outcomes

Our project is designed in accordance with OpenPlant ideas and aimed at development of new tools for plant synthetic biology. Open sharing of standardised resources is an underling principle of the project. Our work will result in a multiscale resource for actin visualization and imaging in living cells, including:

- Two PhytoBricks with actin fluorescent reporter GFP-ABD2-GFP and symbiotic specific promoter. These parts will conform to the common syntax and can be a valuable addition to open-source DNA parts, which can be useful for other researchers.
- The final vector containing transcriptional unit driven by constitutive Ubiquitin promoter (pUBQ:GFP-ABD2-GFP), that is functional in Medicago and many other transformable model plants, and crops.
- A stable transgenic *Medicago truncatua* line expressing GFP-ABD2-GFP actin reporter. This is a valuable resource which will allow the demonstration of actin dynamics and deciphering its role during plant-microbe interactions. Availability of this line enables researchers to cross it with Medicago mutants of actin regulators. It will allow to analyse in details actin distortion caused by mutations and help to our better understanding underlying mechanisms. Moreover, this tool will enable interdisciplinary studies with unrelated microbes (symbionts and pathogens) that share colonization strategies. It will extend our knowledge about the mechanisms of plant interactions with beneficial and detrimental microbes, which are exceptionally valuable for development of new strategies of disease resistance breeding in crops.

To advertise and present available tools to the wider scientific community we will publish project results as a methodological paper with open access.

Our project creates a new collaboration between Cambridge and John Innes Centre. The work will be divided into two parts:

1. Cloning of DNA parts in PhytoBricks and final vector generation. This part will be performed by Aleksandr Gavrin in The Sainsbury laboratory, Cambridge and will take two months' time.
2. *Agrobacterium* mediated Medicago transformations. This work will be done by Prof. Wendy Harwood group in John Innes Centre. Based on their previous experience with Medicago transformation we assume that it will take 6 months.

Therefore, we anticipate that our goals are realistic in a term, which is slightly longer than the allowed time limit. However, we are confident that within the allowed 6 month we can finish the first part and deliver the second part to a stage close to the goal that enables us to write an online report.

Sponsor for the research and cost centre

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I confirm that I have the full support of the sponsor listed above and that they can be added to the OpenPlant Fund mailing list to receive project updates (to which they can unsubscribe at any time).

Budget

Enzymes, primers and other consumables	300£
Orzaez Lab GoldenBraid 2.0 Kit	300£
Medicago transformation	5700£
Total	**6300£**

Additional funding will be provided by core funds available to Sebastian Schornack.

References

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