Project Title:

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

Report Title:

Progress in the project, 6 month report, August 2018

Summary

Actin cytoskeleton and its coordinated dynamics are required for a multitude of plant cellular functions, including growth, development, immunity and symbiotic interactions. The aim of our project is to develop a tool for actin visualization and imaging in living cells of *Medicago truncatula* as a main model plant to study root nodule and arbuscular mycorrhiza symbiosis as well as interactions with different pathogens. The generated tools consist of several universal modules and vectors, which can be used for actin visualization in living cells of plants and their infecting oomycete pathogen and therefore useful to the wide scientific community involved in actin and plant-microbe interaction related research.

Report and outcomes

Our project is aimed at development of new tools for actin visualization and imaging in living cells of *Medicago truncatula*. To do this we decided to use a genetically encoded actin reporter based on the fimbrin Actin Binding Domain 2 (ABD2) flanked by green fluorescent proteins (GFP-ABD2-GFP). This reporter has minimal side effects on plant growth and shows high quality actin labelling. Since our project is designed in accordance with principles of open sharing and standardised resources we generated GFP-ABD2-GFP and two symbiotic specific Leghemoglobin (pLB) promoters as Level 0 Modules for Golden Gate cloning. Then, using these parts we generated several Level 1 modules containing transcriptional units for actin reporter expression under control of Arabidopsis Ubiquitin 10 (pUBQ10) constitutive promoter and LB promoters as well as GUS reporters driven by LB promoters (Table 1). Combining this transcriptional units we generated destination vectors for actin reporter and promoter GUS fusion of LB promotors. All generated modules and destination vectors are conform to the common syntax and may be useful for other researchers studying actin dynamics and its relevance for different plant-microbe interactions. Currently we are testing all our vectors by means of transient expression in Medicago hairy roots. Subsequently, these vectors will be made publicly available at Addgene.

Table 1. List of generated vectors

	Vector	Description
1	pL0M-CS-GFP-ABD2-GFP	Level 0 module containing actin reporter
2	pL0M-PU-pMtLB120-1	Level 0 module containing Medicago LB promoter
3	pL0M-PU-pPsLB	Level 0 module containing pea LB promoter
4	pL1M-R2-pMtLB120-1:GFP-ABD2-GFP	Level 1 module containing actin reporter driven by Medicago LB promoter
5	pL1M-R2-pPsLB:GFP-ABD2-GFP	Level 1 module containing actin reporter driven by pea LB promoter
6	pL1M-R3-pAtUBQ10:GFP-ABD2-GFP	Level 1 module containing actin reporter driven by Arabidopsis Ubiquitin 10 promoter
7	pL1M-R2-pMtLB120-1:GUS	Level 1 module containing GUS reporter driven by Medicago LB promoter
8	pL1M-R3-pPsLB:GUS	Level 1 module containing GUS reporter driven by pea LB promoter
9	pL2V- pMtLB120-1:GUS-pNOS:dsRed	Destination vector containing GUS reporter driven by Medicago LB promoter and dsRed marker
10	pL2V- pPsLB:GUS-pNOS:dsRed	Destination vector containing GUS reporter driven by pea LB promoter and dsRed marker
11	pL2V- pUBQ10: GFP-ABD2-GFP - pPsLB: GFP-ABD2-GFP	Destination vector containing actin reporter driven Arabidopsis ubiquitin 10 promoter and symbiotic LB promoter from *Pisum sativum*
12	pL2V- pUBQ10: GFP-ABD2-GFP - pMtLB: GFP-ABD2-GFP	Destination vector containing actin reporter driven Arabidopsis ubiquitin 10 promoter and symbiotic LB promoter from *Medicago truncatula*
13	pDONR221-P4P1R	Entry vector containing *P. palmivora* UBC2 promoter
14	pDONR221 Lifeact:mScarlet-I	Entry vector containing mScarlet-I tagged actin reporter
15	pDONR221 Lifeact:mCitrine	Entry vector containing mCitrine tagged actin reporter
16	pDONR221-P2RP3	Entry vector containing *P. palmivora* UBC2 terminator
17	pUB1500-Dest Lifeact:mCitrine	Destination vector containing mCitrine tagged actin reporter driven Arabidopsis ubiquitin 10 promoter
18	pTORKm34GW UBC2pro:Lifeact:mScarlet-I	Destination vector containing mScarlet-I tagged actin reporter

The second goal of our project was to generate a Medicago accession R108 stable transgenic line expressing a dual transcriptional unit pUBQ:GFP-ABD2-GFP + pLB:GFP-ABD2-GFP. This would enable actin imaging in all types of cells and tissues during interactions with root and nodule inhabiting microorganisms. However, recently in personal communications with Prof. Elison Blancaflor (Plant Cell Biology Lab, Noble Research Institute, Ardmore, USA) we learnt that his group already generated a Medicago line expressing pUBQ10:GFP-ABD2-GFP. This line has never been published or made publicly available. We therefore established a collaboration with Prof. Blancaflor and currently are designing a collaborative project. Prof. Blancaflor provided us with seeds of this line. We tested it for plant pathogen and symbiotic interactions. This line showed a good extent of actin visualization and is well suited for plant-microbe interaction research (Figure 1A, B). Moreover, a group of Prof. Kong (State Key Laboratory of Plant Genomics, Institute of Microbiology, Beijing, China) just published similar transgenic Medicago line in August 2018. Their line as well expresses the fluorescent actin marker ABD2-GFP driven by UBQ10 promoter (Zhang et al., 2018). Even though the ubiquitin

promoter is not active in fully developed symbiotic cells of Medicago nodules colonised by nitrogen-fixing rhizobia its activity covers most types of plant tissues. Therefore, we consider generation of another Medicago stable transgenic line with our construct as an unjustified spending of resources.

Instead of replicating a Medicago line expressing the actin reporter we focused our efforts on establishing an actin labelled *Phytophthora palmivora*. *P. palmivora* is a devastating phytopathogen, which causes root, bud and fruit rotting diseases in many important crops. Despite its negative impact, nothing is known about the molecular basis underlying its ability to infect its host species, including Medicago (Evangelisti et al., 2017). The dynamics of actin in Medicago-pathogen interactions are not known either. To this end, together with Dr. Edouard Evangelisti, who previously developed a protocol for *P. palmivora* transformation, we designed another actin reporter with two different fluorescent tags Lifeact:mCitrine and Lifeact: mScarlet-I. Lifeact is the second most used actin reporter, which stained filamentous actin structures in eukaryotic cells. Based on that we generated two destination vectors for the actin reporter expression in plants (pUB1500-Dest Lifeact:mCitrine) and in *P. palmivora* (pTORKm34GW UBC2pro:Lifeact:mScarlet-I). The last was successfully introduced into *P. palmivora*. The transformants clearly show actin distribution within growing hyphae without any effects on its virulence (Figure 1C). The strain will be available upon request.

Changes to team

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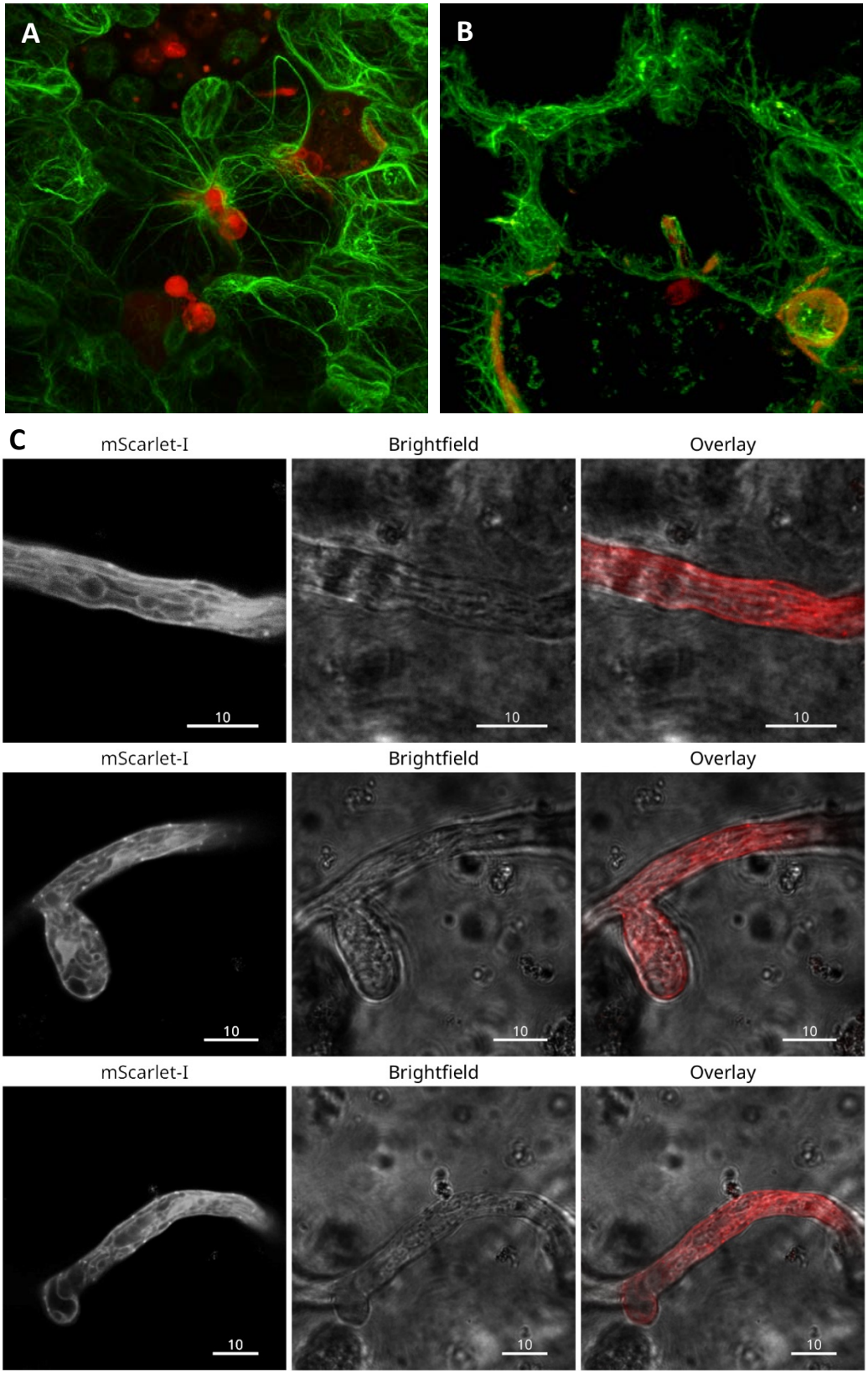

Figure 1. Dynamic rearrangement of actin filaments (GFP) at the epidermal infection sites with *Phytophthora palmivora* (RFP) (A) and around intracellular haustoria of *P. palmivora* (B). Actin labelling in *P. palmivora* expressing Lifeact:mScarlet-I (C).

Expenditure

	Item	Price (£)
1	BsaI-HFv2 NEB (R3733)	48
2	T4 DNA Ligase NEB (M0202S)	46.40
3	BSA NEB (B9000S)	19.20
4	BpiI (BbsI) Life Technologies (ER1011)	20.09
5	QIAprep Spin Miniprep Kit (50) Qiagene (27104)	48.52
6	One Shot™ TOP10 Chemically Competent *E. coli* Invitrogen (C404010)	509.03
7	Gene synthesis GENEWIZ	982.05
8	Horticulture facilities (£0.27 per tray per day)	86.4
9	Confocal microscopy facilities (£35 per hour)	1050
10	One Shot ccdB Survival Competent *E. coli* Invitrogen (A10460)	171.46
11	LR Clonase II Plus enzyme kit Invitrogen (12538120)	379.8
12	Phusion High-Fidelity DNA Polymerase NEB (M0530S)	61.60
13	X-GlcA Sodium Salt 250mg Melford (12954-41-9)	63.50
	TOTAL	3231.53

Follow on Plans

We will not be claiming the £1,000 follow-on fund because we generated vectors and the second part of the original project will not be carried out. We have utilised the funds made available vectors for Medicago and *P. palmivora* labelling of actin. The vectors will be available through Addgene and the transgenic *P. palmivora* isolate will be distributed upon request. Our remaining work in this project will aim at infecting actin labelled roots with actin labelled pathogen to establish whether plant and microbe co-align their filaments when forming intimate structures such as haustoria or whether actin organisation is independent in the host and microbes. The rest of money will be spend for horticulture and confocal microscopy facilities costs, which are necessary for further validation of the generated vectors and the proposed remaining works.

References:

1. Zhang X, Han L, Wang Q, Zhang C, Yu Y, Tian J, Kong Z. The host actin cytoskeleton channels rhizobia release and facilitates symbiosome accommodation during nodulation in *Medicago truncatula*. (2018) New Phytol. doi: 10.1111/nph.15423.
2. Evangelisti E, Gogleva A, Hainaux T, Doumane M, Tulin F, Quan C, Yunusov T, Floch K, Schornack S. Time-resolved dual transcriptomics reveal early induced *Nicotiana benthamiana* root genes and conserved infection-promoting *Phytophthora palmivora* effectors. (2017) BMC Biology 15:39.