Receptor-like kinases at the initiation and maintenance of AM symbiosis

Chai Hao Chiu, Hector Montero, Uta Paszkowski (University of Cambridge)

6-month progress report

Summary

Amongst the key signalling components involved in arbuscular mycorrhizal symbiosis are receptor-like kinases that perceive hitherto unknown extracellular stimuli and transduce these signals into intracellular responses. Of particular interest are lysin-motif (LysM) receptor kinases and a new family of symbiosis-specific receptor kinases, both of which are found in land plants that develop symbiosis with AMF. In our model organism, rice, the LysM receptor kinase *CERK1* is required for the perception and accommodation of both beneficial and pathogenic fungi; whereas the symbiotic-specific kinase *ARK1* regulates the maintenance and longevity of the symbiosis. In addition, both receptor kinases appear to be key signalling nodes in their respective processes, each requiring the concerted action of unknown co-receptors that perceive their respective extracellular stimuli.

We proposed to understand the mechanisms of receptor kinase signalling via two main approaches, both involving DNA synthesis of Golden Gate modules for subsequent cloning and transformations. Creating chimeric receptors and receptor variants is laborious using traditional cloning methods. The Golden Gate modules created would accelerate the assembly, transformation and biological characterisation of symbiotic signalling in roots significantly.

Progress & Outcomes:

1) Synthesis of promoter, coding sequence modules for receptor-like kinases

We synthesized and/or produced the following modules for Golden Gate cloning, with the red items in progress to be received

- pOsCERK1, PU Module
- OsCERK1, S Module
- pOsNFR5, S Module
- OsNFR5, PU Module
- pOsCEBiP, PU Module
- OsCEBiP, S Module
- pOsARK1, PU Module
- pOsARK2, PU Module
- OsARK1, S Module
- OsARK2, S Module

However, in the actual Golden Gate reactions to assemble a transcriptional unit of **pOsCERK1-OsCERK1-GFP-tHSP** and a similar construct for OsNFR5, we failed to obtain a complete Level 1 construct after repeated attempts for two months. We encountered the same problem with transcriptional reporters, also an initial goal. The problem may lie with secondary structures formed by the promoter modules.

As such, we synthesized the full constructs of each receptor-like kinase fused to fluorescent proteins (about £550 each for OsCERK1 and OsNFR5; received). This problem that surfaced early in the first round of cloning also means that the planned scenario of generating chimeric receptors by creating different modules for different domains of receptor kinases is significantly curtailed until the problem in Level 1 Golden Gate reaction is identified and resolved.

Nonetheless, the subsequent plan in response to the problem, is to generate kinase variants as synthesis variants to enable us to dissect their signaling roles. These synthesis orders for variants are currently to be finalized.

2) Transformation and testing of constructs

Of the constructs created so far, we have sent the constructs for transformation into rice plants for subsequent characterization in stable lines. We expect the transgenic lines to arrive in the next two months; and for results from characterizing these lines to emerge in the next 6 months.

The second round of rice transformation will begin when additional modules are received, and their transcriptional units assembled.

3) Tabulation of Expenditure (current and future):

Component	**Spent**	**To be spent (by 2nd week of April)**	To be spent in future
Golden Gate Modules (DNA synthesis & cloning) Red indicate modules to be received	£750 £650		
Full transcriptional units (DNA synthesis)	£1,100	£500 (to generate synthesis variants)	
Consumables (including mainly Sanger sequencing costs to confirm constructs, and a portion towards phenotyping and plant transformation)	£300		£700
Total	**£2,800**	**£500**	**£700**

4) Follow on plans

We intend to continue further experiments to understand the signaling functions of the two key receptor kinases, *CERK1*, *ARK1*; and their potential partner receptor kinases. Despite the obstacles in Golden Gate cloning, we have generated constructs for characterization, firstly in stable transgenic plants. The transformation is ongoing and we expect the lines to be delivered in the next two months. These constructs will further be used for characterization in other transient expression systems in the future. We plan to perform biochemical and cell biology experiments upon confirmation and selection of best lines of transgenic plants. The additional £1000 will be extremely helpful in covering more transformation costs with additional constructs, and with completing the characterization of the plants containing the assembled modules developed from the first initiative.