A receptor-ligand module that triggers cell death in plants: a killer in disguise

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Project Description:
Background - Most of our food is a product of plant reproduction with fertility and seed set critical for crop yield and thus food security. Self-incompatibility (SI) is a genetically controlled system to prevent self-fertilization in 60% of flowering plants, encouraging outbreeding and genetic diversity. For this reason, SI has made a significant contribution to the evolutionary success of flowering plants. After pollination, SI utilizes cell-cell recognition to prevent self-fertilization by inhibition of pollen tube growth, which is crucial for the delivery of sperm cells to the egg cell inside the pistil. This involves a highly specific interaction between a pollen-expressed protein and a cognate pistil protein that results in recognition and inhibition of genetically identical or self-(incompatible) pollen, but not cross (compatible) pollen. The SI system of *Papaver*, which triggers programmed cell death (PCD) in incompatible pollen tubes, is one of the best-studied SI systems and represents a model system for investigating intracellular signalling in plants, particularly in relation to PCD (1). Papaver SI is controlled by a bipartite module comprising two polymorphic S-determinants: A pollen-expressed transmembrane protein, PrpS, interacts with cognate PrsS, a small protein secreted by the stigma, to induce rapid pollen tube growth arrest followed by PCD (2,3). It was recently established that the *Papaver* SI-PCD system can be functionally transferred to Arabidopsis, despite the huge evolutionary distance (~140 million years apart), effectively rendering Arabidopsis self-incompatible (4,5). Even more remarkable, the bipartite PrpS/PrsS module can trigger growth arrest and PCD in sporophytic tissues of Arabidopsis (manuscript under review). This functional transfer of *Papaver* SI to Arabidopsis suggests that PrpS and PrsS could act as a 'lock and key' mechanism that once in place can recruit similar signalling pathways across highly divergent species. Therefore, the nature of pollen PrpS and its interaction with PrsS is of considerable interest.

Aim - Despite a good understanding of the signaling events leading to growth arrest and PCD, the mechanistic basis of how the PrpS/PrsS module functions is still unknown. PrpS is a plasma-membrane protein with no homologues in databases. We hypothesise that PrpS functions as a ligand-gated ion channel. The project aims to identify the functional nature of PrpS and establish the dynamics of PrpS-PrsS receptor-ligand interactions.

Methodology - This project provides an exciting opportunity for a talented and motivated student to study the nature of this fascinating receptor-ligand module using the following multidisciplinary approach:

1. Establish the basis for the specificity of the PrpS-PrsS interaction using targeted site-directed mutagenesis. The student will perform structural modelling of PrpS proteins as well as predicting the likely interactions between cognate PrpS and PrsS proteins. These *in silico* studies will form the basis for the functional analysis of specific modifications.
2. Determine if PrpS is indeed an ion channel. The student will use patch-clamp electrophysiology to establish if PrpS acts as a channel.
3. Establish if PrpS forms a multimeric complex. The student will use biochemical and state-of-the-art live cell imaging techniques to determine if PrpS forms oligomers and if its oligomeric state is dependent on

interaction with its cognate ligand.

Impact - Although fundamental by nature, the outcomes of this collaborative project will underpin the ability to breed hybrid crops – plants whose 'hybrid vigour' gives them better yield and strength than their parents. In addition, PCD constitutes a source of unexplored molecular targets for new herbicidal modes of action. Functional knowledge about SI-PCD can therefore lead to the development of smart herbicides.

Supervisory team - The student will be supervised by a multidisciplinary team with all the expertise to achieve the goals set out for this DTP studentship. The lead supervisor is Maurice Bosch (IBERS, Aberystwyth University), wo is a molecular cell biologist with a longstanding interest and track record in studying plant reproduction. The project will be co-supervised by Dr Liam McGuffin and Professor Gary Stephens (both University of Reading). Liam McGuffin is an expert on the development of computational algorithms for modelling protein structures, functions and interactions. Gary Stephens is an electrophysiologist with expertise in ion channel and ligand-gated ionotropic receptor physiology.

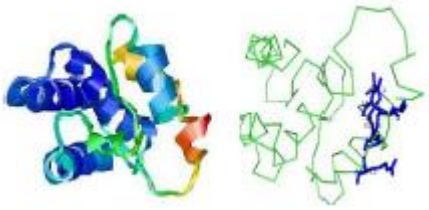

Figure 1 Left: Model of PrpS1, blue (high accuracy) through green, yellow and orange to red (low accuracy). Right: Predicted ligand binding residues are shown as blue sticks

References:

1. Wang et al (2019) J Exp Bot 70:2113; 2. Thomas & Franklin-Tong (2004) Nature 429:305; 3. Bosch & Franklin-Tong (2007) PNAS 104:18327; 4. de Graaf et al (2012) Curr Biol 22:154; 5. Lin et al (2015) Science 350:684.