Abstract

Food supply and security are urgent issues in a world of increasing population. Plants, which provide almost all of the world food supply, are under constant attack by various types of microbial invaders, thus posing tremendous threats to global food security. Of these microbes, rust fungi, a diverse group of obligate biotrophic phytopathogens having over 7,000 species, are causal agents of some devastating plant diseases, responsible for significant yield losses of several important crop species. Among the rust fungi, flax rust (*Melampsora lini*), the cause of rust disease to flax cultivars (*Linum usitatissimum*), is of great research curiosity more so from a scientific perspective and to a lesser extent economic. As an obligate biotroph, the flax rust fungus requires a living host, and hence it has to contend with the host's defence machinery to establish a successful infection and absorb nutrients from the infected host before sporulation. To protect themselves, plants devote a large proportion of their genome to the recognition of effector molecules secreted into the plant cells by the invading pathogen. This recognition and defence activation is orchestrated by disease resistance or R proteins. But the pathogen effector molecules show low sequence homology to other known proteins, making it difficult to predict their role in the infection process. For many years, flax and flax rust have been used as a model system for studying rust infection and disease resistance. The experiments described in this thesis aim to identify the crucial residues involved in recognition specificity of the flax rust effector AvrM and elucidate the roles of these residues in the interaction with the M flax resistance protein.

The *AvrM* effector locus of the flax rust fungus encodes six variants, designated *AvrM A-E* and *avrM*. Published structural and biophysical analysis of the AvrM-A protein predicts that it exists as a stable homodimer, forming a unique negatively charged pocket at the dimer interface, but not found in the similar region of avrM (Ve, 2011; Ve et al., 2011). Previous research has demonstrated that AvrM-A is recognised by, and interacts with, the M flax rust resistance protein, but that avrM's lack of recognition and interaction is limited to a region containing 13 polymorphic residues between AvrM-A and avrM (Catanzariti et al., 2010). Chapter 3 of this thesis describes a mutation analysis, coupled with an *in planta* hypersensitive response (HR) assay, showing that no single polymorphic residue controls this recognition event. Results presented in Chapter 4 demonstrate that a combined mutant of

three polymorphic residues in avrM, when changed to their AvrM-A counterparts, enable partial M recognition, and the addition of another mutation enables full recognition. On the contrary, the same polymorphic substitutions, as in avrM, were also tested in the avirulence protein, AvrM-A, which is recognized by M, and found that multiple reciprocal substitutions (up to a quadruple mutant) did not prevent recognition by M. This suggests that other residues in AvrM-A still provide enough support to induce M-activated HR.

Furthermore, three non-polymorphic charged residues, which collectively form a negatively charged pocket at the interface of the AvrM-A dimer, when substituted for alanine, neutralize the charged pocket and thus prevent M interaction and recognition. Yeast-Two Hybrid (Y2H) assay confirmed that avrM does not, but the AvrM-A interacts with the M flax resistance protein. Size-exclusion chromatography (SEC) coupled with Multi-angle Light Scattering (MALS) and Small-angle X-ray Scattering (SAXS) show that AvrM-A is a stable dimer in solution, but avrM is a monomer. By the comparison of these results, the AvrM-A mutants that abolished HR, showed no interaction in the Y2H assay, but they are still dimeric as determined by SEC-SAXS analysis. On the other hand, the gain-of-recognition mutants of avrM could not be shown to interact with the M protein in the Y2H assay, but were still dimers as revealed by the SEC-MALS and SEC-SAXS analyses.

Collectively, these data suggest that for AvrM effector molecules to be recognisable by M, they must form homo-dimers. Also, the negatively charged pocket at the dimer interface of AvrM-A protein facilitates interaction with, and activation of, the M protein. Alteration of the quaternary structure of an effector protein represents another way in which a pathogen can avoid recognition by the plant innate immune system. This research has unravelled how the AvrM protein escapes M detection and will help guide further research aimed to understand how the effector molecules function to aid the pathogen, and how host R proteins detect them and protect the plant.