The molecular bases of recognition of the M. Oryzae effector protein AVR-Pia by the rice immune receptor RGA5

Diana Ortiz, Karine de Guillen, Véronique Chalvon, André Padilla, Thomas Kroj

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Plant immune receptors of the NLR class are multi domain proteins characterized by an N-terminal TIR or coiled-coil domain, a central nucleotide-binding domain and an N-terminal leucine-rich domain. NLRs act by recognizing pathogen effector proteins in the plant cytosol either by direct binding or in an indirect manner. Despite the cloning of the first plant NLRs more than 20 years ago, the molecular bases of effector recognition remain badly defined. Here we used a structure-aided approach to elucidate the molecular recognition mechanisms of the AVR-Pia effector protein from the blast fungus Magnaportha oryzae by its cognate NLR receptor RGA5 from rice. AVR-Pia binds directly to an uncommon C-terminal domain of RGA5 that is homologous to the copper chaperone ATX1 (Related to ATX1 domain or RATX1 domain). By using recombinant AVR-Pia and ATX1 proteins, the affinity of binding was determinate by in vitro binding experiments and the AVR-Pia binding surface was delimitated by NMR titration experiments. Yeast two hybrid and in planta protein-protein interaction studies with AVR-Pia mutant proteins confirmed this interaction surface and identified amino acids of AVR-Pia that are crucial for RATX1 binding. The importance of these amino acids for effector recognition during rice infection was confirmed with transgenic M. oryzae isolates expressing AVR-Pia mutant variants. This study sheds new light on NLR function and opens the way to a molecular understanding of effector recognition in cereals.