Identifying amino acid residues conferring the functional differences in immune proteases Rcr3 and Pip1 of tomato in triggering hypersensitive cell death

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Bursary Report

Cladosporium fulvum is a fungal pathogen that causes tomato leaf mould. It secretes the effector AVR2, which is perceived by the tomato papain-like cysteine protease RCR3 in combination with the receptor-like protein Cf-2, and triggers the hypersensitive response (HR), a plant immune response based on programmed cell death. PIP1 is closely related to RCR3 and can interact with AVR2 but does not trigger HR in the presence of AVR2. This provides an excellent model system to study the way in which plants interact with pathogens.

Over eight weeks in the summer I worked in the lab of Professor Renier van der Hoorn at the Department of Plant Sciences, Oxford University, to investigate which residues in RCR3 are important for triggering HR, presumably by interacting with Cf-2.

Firstly, we developed a quantitative HR assay based on ion leakage for quantifying cell death in *Nicotiana benthamiana* leaves transiently over-expressing AVR2, Cf-2 and RCR3. This was to supplement the qualitative HR assay used so far, which involved binary scoring visual tissue collapse, but which couldn't distinguish the quantitative effects played by individual residues.

Previous domain-swap by Jiorgos Kourelis had shown that, having divided RCR3 and PIP1 into six arbitrary parts, part 3, 5 and 6 of RCR3 were important for triggering HR. I focused on parts 3 and 6 over the course of my time in the lab. We used Golden Gate cloning to produce hybrid versions of PIP1 and RCR3 containing increasingly larger proportions of PIP1 to identify individual residues in part 3 and 6 important for HR induction. These constructs were then tested in the ion leakage assay, and compared to RCR3 and PIP1. I was able to identify two residues in part 3 and six residues in part 6 that are important in triggering HR. Further work will focus on identifying important residues in part 5 using the quantitative assay to quantify the relative importance of the different residues.

Aside from shedding more light on the mechanism by which tomato perceives a fungal pathogen, this research has potential broader practical implications. This project could lead to the engineering of PIP1 to interact with Cf-2 and this could be used to develop resistance to multiple pathogens that secrete unrelated PIP1 inhibitors.

Over the course of the eight weeks I have learned a lot about the process of research, including the challenges of doing large-scale experiments as well as the satisfaction of getting new results. I also increased my knowledge on techniques like molecular cloning and Activity-based Protein Profiling (ABPP). I would like to thank the BSPP for providing me with the funding to allow me to take this opportunity, and all the members of the Renier van der Hoorn lab (particularly Jiorgos Kourelis) for all of their help and encouragement, as well for making the lab a fun and stimulating place to work in over the summer.

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