In planta silencing with short antisense oligodeoxynucleotides as a new tool to confirm fungal virulence factors of the obligate pathogen barley powdery mildew

This summer I was very privileged to be able to work under the supervision of Dr Bindschedler, in the plant pathology lab at Royal Holloway, University of London. The aim of the project was to investigate the efficiency of using the novel methodology of Host Induced Gene silencing (HIGS) to silence effectors and housekeeping genes of the obligate fungal pathogen *Blumeria graminis* f.sp.*hordei*, the causal agent of barley (*Hordeum vulgare*) powdery mildew.

This new HIGS method involves using small antisense oligodeoxynucleotides (ODNs, 19-25 mers) which are delivered into excised barley leaves via transpiration to silence effectors of *Blumeria*. *In planta* delivery of 6FAM-labelled ODNs has been evidenced by fluorescence microscopy driven by transpiration up the vascular tissue into the cytoplasm of plant cells. It is proposed that the ODNs are then translocated to *Blumeria* tissues via the haustoria plant-fungus interface. Haustoria are structures typical to biotrophic fungi, and are involved in nutrient uptake from the host and effectors delivery in the host. It is also assumed that the antisense ODNs trigger RNAse-H dependent degradation of the targeted mRNA, directing the targeted mRNA to the RNAi silencing complex, ultimately causing mRNA degradation. Alternatively silencing might be the result of translational arrest.

My work was primarily involved in *in planta* silencing of the phytopathogen effector BEC1011 in different barley cultivars. BEC1011 is a haustoria specific ribonuclease-like effector predicted to be secreted to the cytoplasm of the plant epidermal cells. I was able to confirm that silencing of BEC1011 with the new HIGS method using a specific phosphorothioate modified oligonucleotide (PTO) targeting BEC1011 (PTO11) led to reduced fungal virulence. Fungal virulence was monitored microscopically by scoring the percentage of successful infection events, as measured by the proportion of conidia producing secondary hyphae, 2 days post infection (2 dpi). My results confirmed that targeting BEC1011 with the PTO11 antisense oligo led to a highly significant reduction in infection in the Golden Promise (GP) cultivar in comparison to the mock control. For the Morex, Ingrid and Maris Otter cultivars, a general reduction in infection following the PTO11 treatment was observed, and was occasionally significant, however this trend couldn't be confirmed in all repeats as statistically significant. This might be partly explained by some cultivar having shown signs of stress (chlorosis). Chlorosis was not observed in Golden Promise leaves. Repeats will be required to be able to draw any conclusion. An alternative plausible explanation is that BEC1011, which is a species specific effector, does have a stronger impact on virulence for only a narrow range of barley cultivars.

Since effectors are more likely to be cultivar specific for virulence, I employed the HIGS methodology for silencing the two powdery mildew housekeeping genes actin and glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Unlike BEC1011 these genes are not Haustoria-specific genes but are genes expressed constitutively throughout the fungus. When either GAPDH or actin were targeted with specific silencing oligos, a statistically significant reduction in infection was observed when compared to the mock control, as estimated microscopically at 2 dpi. Furthermore, reduced relative fungal biomass and *Blumeria* GAPDH/ actin transcripts levels in the host (estimated by qRT-PCR) could be correlated with the reduced virulence phenotype observed microscopically.

I also performed some preliminary experiments, testing the effect of silencing a barley pathogen related protein 5 (PR5) isoform, which is known to interact with the *Blumeria* effector BEC1054. I had to design the silencing oligos, first retrieving the correct CDS for the Golden Promise cultivar to submit it to a RNAi specific designing software. Selection of the silencing 19-mer oligomer took into consideration the probability score for silencing efficiency, and avoiding off-target silencing of genes showing homology matches in barley or *Blumeria* (Blast search for validation). It was hypothesised that silencing of this PR5 would lead to decreased resistance, as PR proteins have historically been associated with the set-up of resistance, expressed usually more quickly and strongly in resistant plants in comparison to susceptible ones. My results first confirmed previous work performed in Dr Bindschedler lab. That is: treatments with 2 specific PTOs to target this PR5 isoform, showed a reduction in fungal infection at 2 dpi (% Hyphae, microscopy). For the first time I could establish that the decreased host susceptibility (microscopy) correlated with reduced fungal biomass (qRT-PCR) as well as a decrease in PR5 transcript levels (qRT-PCR), thus confirming

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that the decreased susceptibility phenotype was associated with PR5 silencing. These rather surprising results need repeating, but certainly open up a new line of exciting investigations.

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