Investigating the Role of Protein Kinase C in *Magnaporthe oryzae*

Submitted by Tina J. Penn

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I certify that all material in this thesis which is not my own work has been identified and that no material has been previously submitted and approved for the award of a degree by this or any other University.

Tina J. Penn

Abstract

Fungi are able to perceive and respond to changes in their environment through the activation of signal transduction pathways. Protein kinase C is a serine/threonine kinase found in all eukaryotes that is implicated in the regulation of signalling pathways. Here I report the identification of a protein kinase C-encoding gene, *PKC1*, which I have shown is cytoplasmically localised and expressed during conidial germination and appressorium formation in the rice blast fungus *Magnaporthe oryzae*. Targeted gene deletion of *PKC1* was attempted unsuccessfully, therefore analysis of Pkc1 was carried out by RNAi-mediated gene silencing and selective kinase inhibition. A hairpin dsRNA–expressing construct was designed to target *PKC1* and was introduced into *Magnaporthe oryzae* under the control of an inducible promoter. Silencing was variable but resulted in a loss of viability and when coupled with failure to obtain a gene replacement, suggests that Pkc1 is essential for viability. *PKC1* gene silencing resulted in a severe hyphal growth defect and reduction in conidiogenesis. The phenotype was rescued by targeted gene deletion of the dicer-like gene, *MDL2*, the result is therefore consistent with gene silencing. A chemical genetics approach to selective kinase inhibition was also attempted. A previously characterised, non-essential kinase with a readily scorable phenoype, *ATG1,* was initially targeted to provide proof of concept in *M. oryzae*. Mutation of an amino acid residue in the ATP-binding site of the kinase resulted in increased susceptibility to chemically modified inhibitors. However, *PKC1* does not tolerate the necessary modification to the ATP-binding site. Finally, analysis of the cell integrity pathway suggests *PKC1* acts on this pathway but constitutive activation of the pathway only partially restored viability to the *PKC1*-gene silenced mutant and it is therefore likely to have other additional cellular targets.