

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

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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, *PKCI*, 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 *PKCI* 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 *PKCI* 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. *PKCI* 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 phenotype, *ATGI*, 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, *PKCI* does not tolerate the necessary modification to the ATP-binding site. Finally, analysis of the cell integrity pathway suggests *PKCI* acts on this pathway but constitutive activation of the pathway only partially restored viability to the *PKCI*-gene silenced mutant and it is therefore likely to have other additional cellular targets.

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