Investigating the role of lipid mobilisation and metabolism in the rice blast fungus *Magnaporthe oryzae*

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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.

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Abstract

The rice blast fungus *Magnaporthe oryzae* infects plants by developing a specialised infection structure known as an appressorium. In *M. oryzae* the appressorium is a melanin-pigmented cell with a reinforced cell wall, allowing the cell to generate enormous internal turgor to enable penetration of the plant tissue by a narrow penetration hypha. Previously it has been shown that mobilisation of lipid droplets to the nascent appressorium is essential for successful plant infection. In this thesis, I describe a series of studies that have identified and characterised genes associated with infection-associated lipid metabolism in *M. oryzae*, including the role of fatty acid β-oxidation, acetyl-CoA transport and metabolism and regulation of lipid body breakdown. First, I report identification of *FAR1* and *FAR2*, which encode putative Zn2-Cys6 binuclear proteins that appear to act as transcriptional regulators of lipid metabolism. Deletion mutants of *M. oryzae FAR1* and *FAR2* were deficient in growth on long chain fatty acids. In addition Δ*far1* mutants were unable to grow on acetate as a sole carbon source. *FAR1* and *FAR2* affect the expression of genes involved in fatty acid β-oxidation, acetyl-CoA translocation, peroxisomal biogenesis, the glyoxylate cycle and acetyl-CoA synthesis. Next, I functionally characterized the *CAR1*, *CAR2*, *CAR3* and *CAR4* genes, which encode enzymes involved in carnitine biosynthesis, which is required for translocation of acetyl-CoA between mitochondria, peroxisomes and the cytoplasm. Only a sub-set of carnitine biosynthetic enzymes was necessary for growth on fatty acids and lipids by *M. oryzae*, but redundancy was also apparent in carnitine biosynthesis, because *CAR1, CAR2, CAR3* and *CAR4* were dispensable for pathogenicity, while the carnitine acetyltranferase, *PTH2*, is essential for rice blast disease. To investigate the role of the appressorium acetyl-CoA pool in more detail, I functionally characterized the acetyl-CoA synthetase gene, *ACS2* and *ACS3*, and *CRC1*, which encodes the mitochondrial carnitine carrier, both of which are highly expressed during appressorium development and appear to play a role in appressorium physiology. Finally, to understand the onset of lipid droplet degradation in more detail, I characterised a putative perilipin, encoded by *CAP20*, which localizes specifically to the periphery of lipid droplets. Perilipins are known to play roles in lipid droplet mobilisation and lipase accessibility. Consistent with this idea, *M.* oryzae mutants lacking *CAP20*, were severely affected in fungal virulence due to impaired appressorium function. When considered together, the results presented in this thesis suggest that lipid body mobilisation and acetyl-CoA metabolism are fundamental processes required for appressoria to function correctly and cause rice blast disease.

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