Epigenetic regulation of *Epichloë festucae* secondary metabolite biosynthesis and symbiotic interaction with *Lolium perenne*

A thesis presented in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

in

Genetics

at Massey University, Palmerston North

New Zealand

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2017

Abstract

Histone methylation is one of several epigenetic layers for transcriptional regulation. Most studies on the importance of this histone modification in regulating fungal secondary metabolite gene expression and pathogenicity have focussed on the role of histone methyltransferases, while few studies have focussed on the role of histone demethylases that catalyse the reversal of the modification. *Epichloë festucae* (Ascomycota) is an endophyte that forms a mutualistic interaction with perennial ryegrass. The fungus contributes to the symbiosis by the production of several classes of secondary metabolites, these have anti-insect and/or anti-mammalian activity. The *EAS* and *LTM* clusters in *E. festucae* are located subtelomerically and contain the biosynthetic genes for two of these important metabolites which are only synthesised *in planta*. Thus, in the host plant these genes are highly expressed, but they are tightly silenced in culture conditions. Previous study has shown that histone H3K9 and H3K27 methylation and their corresponding histone methyltransferases are important for this process. In this study, the role of histone lysine demethylases (KDMs) in regulating these genes and the symbiotic interaction is described.

Eight candidate histone demethylases (Jmj1-Jmj8) were identified in *E. festucae*, among these proteins are homologues of mammalian KDM4, KDM5, KDM8, JMDJ7, and *N. crassa* Dmm-1. The genes for the proteins were overexpressed in *E. festucae* and histone methylation levels were determined in the strains. Overexpression of the genes was not observed to cause any change to the culture and symbiotic phenotypes of the fungus. Western blot analysis subsequently identified one of the proteins, KdmB, as the histone H3K4me3 demethylase. Further analysis by ChIP- and RT-qPCR showed that demethylation of H3K4me3 by KdmB at the *eas*/*ltm* genes is crucial for the activation of these genes *in planta*. The full expression of several other telomeric genes was similarly found to require KdmB. On the other hand, the COMPASS H3K4 methyltransferase complex subunit CclA that is required for H3K4 trimethylation in *E. festucae* represses the *eas*/*ltm* genes in culture conditions by maintaining H3K4me3 levels at the loci. Thus, these findings suggest a repressive role for H3K4me3 at these subtelomeric secondary metabolite loci and are consistent with the role of H3K4me3 in yeast telomeric silencing. Disruption of *kdmB* did not affect the symbiotic interaction of *E. festucae* with the host grass but severely reduced the levels of lolitrem B, an animal neurotoxin. At the same time, the levels of ergovaline, another animal toxin, and peramine, an insect feeding deterrent, were not affected. Therefore, disruption or inhibition of KdmB may also serve as a promising approach for future endophyte improvement programmes.

The *E. festucae* homologue of KDM8 (an H3K36me2 demethylase), Jmj4, was further investigated in this study but no H3K6 demethylase activity was found for the protein. Both disruption and overexpression of the gene encoding Jmj4 similarly had no effect on the culture and symbiotic phenotypes of *E. festucae*. However, deletion of *setB*, encoding the homologue of yeast Set2 (H3K36 methyltransferase) specifically reduced histone H3K36me3 levels in *E. festucae*. This contrasts with deletion of Set2 in other fungi which affected H3K36 mono-, di- and trimethylation. The Δ*setB* mutant was severely impeded in development, and was unable to establish infection of the host plant. Introduction of the wild-type *setB* gene reversed these phenotypes.

This study shows that H3K4 trimethylation controlled by CclA and KdmB is an important regulator of subtelomeric secondary metabolite genes in *E. festucae* but not for the symbiotic interaction of the fungus with perennial ryegrass. On the other hand, the histone H3K36 methyltransferase SetB specifically

controls H3K36 trimethylation in *E. festucae* and is required for normal vegetative growth and ability of the fungus to infect the host plant.

Acknowledgements

My sincerest gratitude to my MSc and PhD supervisor Professor Barry Scott who has kindly given me the opportunity to undergo my scientific training in his lab. Thank you for your incredible support throughout my studies, for the guidance but also the freedom to develop my ideas. I appreciate the generous support given towards attending conferences, the opportunity to be involved in side projects and am grateful for your enthusiasm in training us all to be good scientists.

A big thank you to my co-supervisors; Dr Tetsuya Chujo for his advice and technical training especially during my MSc years, to Dr Linda Johnson for the constant support and helpful directions for my research project, and to Dr Tracy Hale for the helpful discussions, technical training, and also for kindly allowing me to conduct the mammalian experiments in her lab. The *cclA* mutants used in this study were also generated by Dr Chujo.

To all the wonderful people in Scottbase, past and present, thank you for the support, discussions and ideas. Thanks also to Dr Pranav Chettri for the many helpful discussions and advice, to Dr Sarah Bond for spending considerable time with training on cell culture and immunofluorescence, to Dr Jeong Park for helpful discussions with ChIP and to Prof Derek White for valuable advice on the plant biology aspects. Thanks to Dr Pierre-Yves Dupont and Dr David Winter for helpful discussions and assistance on the statistics and bioinformatics. And thanks also to Wade Mace (AgResearch) for the alkaloid analysis. Finally, to Dr Mac Campbell for the invaluable discussions on… trout, and the awesome times on the river – this should be higher up!

I would also like to acknowledge the financial support provided by the AgResearch PhD scholarship. All imaging experiments in this study were performed at the Manawatu Microscopy and Imaging Centre (MMIC).

Finally, thanks to Monica, Chelsea and my parents for the love, support and encouragement.

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