Developing RNAi in the scabies mite, Sarcoptes scabiei

2014

Arnold Hitchcock Travel Award

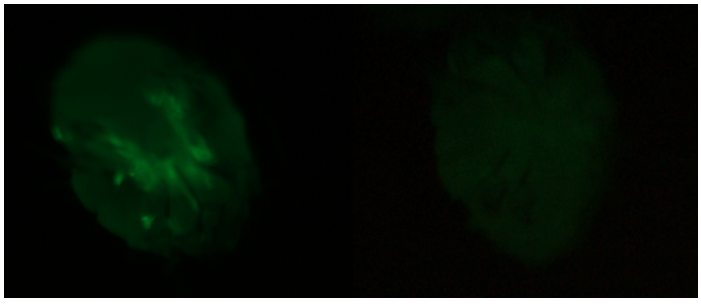
The Arnold Hitchcock Travel Award of £500 generously awarded by the Perry Foundation provided me with a unique opportunity to visit the Bacterial Pathogenesis and Scabies Laboratory in the Queensland Institute of Medical Research Berghofer Medical Research Institute (QIMRB, Brisbane, Australia) to complete a 6 week research project, 'Developing RNAi in the scabies mite, *Sarcoptes scabiei'*.

Introduction to the project

Scabies, caused by the ectoparasitic mite *Sarcoptes scabiei*, is a zoonotic problem on a worldwide scale, with an estimated 300 million people infested (1). With the threat of drug resistant *S. scabiei* emerging, novel control strategies must be sought (2). Sheep scab, caused by the ectoparasitic mite *Psoroptes ovis*, is a significant economic burden (3) and poses a serious welfare concern due to the severe symptoms (4). Similarly, the current control measures are considered unsustainable, thus alternative treatments are required. By understanding the underlying molecular biology of these mites in greater detail and identifying gene candidates that are important in the development of acaricidal resistance through RNA interference (RNAi) studies, new compounds or vaccines can then be developed to target those candidates. My PhD project aims to develop and ultimately use RNAi for selective gene knockdown and vaccine candidate identification in the ectoparasitic mite *P. ovis.* This would enable screening for candidate antigens in both a time and cost effective manner. The purpose of my research visit to the QIMRB was to assess whether or not RNAi could also be applied to *S. scabiei*.

RNA interference in context

RNA interference (RNAi) is an evolutionarily ancient mechanism of gene regulation, whereby silencing of specific gene expression is achieved following introduction of complementary double stranded RNA (dsRNA) to the target gene's messenger RNA sequence. The methodology, in parasitic mites, was pioneered in *Varroa destructor* (5) and my PhD studies are focussed on the development of an RNAi screen in the sheep scab mite (*P. ovis*) using similar methodology. Immersion of *Varroa* mites in solution containing dsRNA specific for the *V. destructor* glutathione S-transferase (GST) mu-class 1 gene (*Vd*GST-mu1) resulted in effective silencing of *Vd*GST-mu1 at both the transcriptional and translational level and therefore represented a promising initial target for establishing an RNAi screen in the scabies mite (5). Furthermore, GSTs have been previously characterised in *P. ovis* (6) and *S. scabiei* (7) and are implicated in the development of acaricidal resistance (8).



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Promising results

The uptake of interfering RNA via the immersion method was demonstrated following overnight incubation at 4°C of mites immersed in either fluorescently labelled siRNA or 0.9%NaCl buffer alone. Thus, indicating that the mites were amenable to this method of achieving dsRNA uptake (Figure 1). Subsequently, dsRNA encoding a *S. scabiei* mu-class 1 GST (*Ss*GST-mu1) and a control gene, Pso o 2, were produced. *Sarcoptes* mites were harvested from a scabies pig infestation model (Figure 2) and immersed overnight with dsRNA encoding either *Ss*GST-mu1 or the control Pso o 2, or 0.9% NaCl buffer alone. Total RNA was extracted from the mites and cDNA generated for quantitative real time PCR (qPCR) to determine gene knockdown based on the relative levels of mRNA in the samples corresponding to the target *Ss*GST-mu1. Preliminary qPCR analyses suggest gene silencing for *Ss*GST-mu1 had been achieved, providing the first demonstration of RNAi in the scabies mite – with the caveat that these analyses are currently undergoing optimisation, for validation.

Final thoughts

The results of this preliminary RNAi study in scabies mites have highlighted some key areas for further development, indeed this is something the Bacterial Pathogenesis and Scabies Laboratory in the QIMRB are keen to pursue, with myself and my supervisors continuing to play an active role. Not only did I gain valuable experience from living and working in a stimulating new environment in a different country, but the variety of challenges that I faced along the way have served to harden my resolve and focus my research at MRI, and will undoubtedly be of huge benefit in the pursuit of my ambition of a career in research after completion of my PhD. I gained valuable mite handling experience and was able to transfer the techniques and skills required for RNAi in mites to the scabies group allowing them to continue this body of work in

my absence. This was achieved through a combination of presentation, demonstration and discussion.

Acknowledgments
I would like to thank the Perry Foundation for awarding me an Arnold Hitchcock Travel Award, my supervisors and friends at the Moredun Research Institute and Royal (Dick) School of Veterinary Studies and similarly, all those involved from the QIMRB in Brisbane for making this research visit such an enjoyable and productive experience.

Links to the host institutes
Moredun Research Institute
http://www.moredun.org.uk

Queensland Institute of Medical Research Berghofer Medical Research Institute
http://www.qimrberghofer.edu.au

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