Functional analysis of the insulin/IGF signalling pathway and the infective larva developmental switch in *Parastrongyloides trichosuri*

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

Doctor of Philosophy
In
Molecular Genetics

at Massey University, Palmerston North,
New Zealand.

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2010

ABSTRACT

Parasitism, in nematodes, is a very successful life strategy which has evolved throughout the Nematoda phylum in several independent events. However, the genetic basis for parasitism remains unknown. *Parastrongyloides trichosuri* is a facultative parasitic nematode of the Australian brushtail possum. This parasite has retained the unusual ability to sample its environment at each generation, and make the developmental decision to develop either into a free-living nematode or, in response to environmental stress, develop into an infective larva, which must then seek out a host in order to complete its life cycle.

The nematode model organism, *Caenorhabditis elegans*, also responds to environmental stresses by developing into a dauer larvae. The dauer hypothesis proposes that dauer larvae and infective larvae are homologous and that dauer larvae may be an evolutionary pre-adaptation that facilitated the evolution of parasitism in nematodes. One of the signalling pathways which control dauer larva development in *C. elegans* is the Insulin/IGF signalling pathway.

Gene orthologues of the insulin/IGF signalling pathway were cloned from *P. trichosuri*: the *daf-2* tyrosine kinase receptor, the *age-1* phosphatidylinositol 3′ kinase and the *daf-16* FOXO forkhead transcription factor. The expression profiles of these genes were characterized by q-PCR which determined that they were differentially expressed during the developmental switch to infective larva. Rescue by complementation showed that a *P. trichosuri daf-16* transgene was able to recover both stress and developmental phenotypes in *C. elegans daf* mutants, suggesting it might perform an orthologous role in *P. trichosuri*.

This research also demonstrated that the biology of *P. trichosuri* infective larvae and *C. elegans* dauer larvae are quite similar. Some of the environmental signals which control the free-living/infective larva developmental switch in *P. trichosuri* were characterized in this study and found to be similar to the environmental signals which trigger dauer larval development. These are: population density, food availability and temperature.

There is a genetic component to the ability to respond to the environmental signals and inbred lines which display diverse developmental plasticity were isolated.

ACKNOWLEDGEMENTS

With much appreciation, I would like to thank the following people; first and foremost, Dr. Warwick Grant, my supervisor, for his support and willingness to let me explore unusual avenues in order to solve problems and satisfy my curiosity. Thank you also to my co-supervisor Max Scott, for all his practical advice and support during my time at Massey.

The following people of the "Worm Lab" team contributed in some very practical and hands on ways for which I am very grateful. Kirsten Grant for worm microinjections, Mark Ralston and Robyn Hirst for the animal husbandry of the possums, Jan Newton-Howes for collaboration on some of the RNAi and quantitative RT-PCR work, Matt Crook, Tina Englbrecht and Marleen Richter for collaborating on the *P. trichosuri* chemical mutagenesis screening and to the AgResearch statisticians and bioinformaticians: Fred Potter, John Koolaard and Roger Moraga Martinez.

I am deeply grateful for the helpful discussions and moral support of Jan Newton-Howes, Jacqui Knight and all the members of the 'Worm Lab" team. And to all my family and friends, who eventually stopped asking: "is it done yet?" and continued to offer their support and love. Finally, thanks to the *P. trichosuri* larvae: several hundred thousand gave up their lives in the biological assays, in order to ensure that the frontiers of science could be pushed back.

I would also like to thank both my employer AgResearch and the FoRST Enterprise Scholarship and the Royal Society Marsden Fund for their financial support throughout this PhD. Approval for the experiments described in this thesis was granted by the Wallaceville and Grasslands Animal Ethics committee AEC # 273.

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ABBREVIATIONS

Abbreviations:

aa	amino acid
BAC	bacterial conditioned medium
BLAST	Basic Local Alignment Search Tool
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bp	base pair
^{o}C	degrees Celsius
cDNA	copy deoxyribonucleic acid
cds	coding sequence
CM	conditioned medium
DMSO	dimethyl sulfoxide
daf	dauer formation
DNA	deoxyribonucleic acid
E. coli	*Escherichia coli*
F_1	First generation
F_2	Second generation
FCS	Foetal Calf Serum
g	gram
gfp	green fluorescent protein
hr	hour
HCl	hydrochloric acid
IIS	Insulin/IGF Signalling
iL3	infective larva
kb	kilobase-pairs
kD	kilodalton
KOAc	potassium acetate
L	litre
L1	first larval stage
L2	second larval stage
L4	fourth larval stage

LD_{50}	Lethal dose at which there is 50% survival
LC_{50}	Lethal concentration, point at which there is 50% survival
LB	Luria-Bertani media
M	molar, moles per litre
mg	milligram
µL	microlitre
mL	millilitre
Milli-Q water	water purified by Milli-Q ion exchange column
µM	micromolar, micromoles per litre
mM	millimolar, millimoles per litre
mRNA	messenger ribonucleic acid
NaOAc	sodium acetate
NGM	Nematode Growth Medium
nmol	nanomole
nt	nucleotide
NTC	No template control
ORF	open reading frame
PCR	polymerase chain reaction
pH	-Log $[H^+]$
PI3′K	Phosphatidylinositol 3′ kinase
P_0	Parental generation
q-PCR	quantitative reverse transcriptase PCR
RACE	Rapid amplification of cDNA ends
RNA	ribonucleic acid
RNase	ribonuclease
RNAi	RNA interference
RT-PCR	reverse transcription-polymerase chain reaction
rpm	revolutions per minute
SDS	Sodium dodecyl sulphate
SL1	Splice leader one
SL2	Splice leader two
5′ or 3′ UTR	5′ or 3′ untranslated region
ts	temperature sensitive