Transcriptome characterisation of cercariae and skin-stage schistosomula in the parasitic helminth *Schistosoma mansoni*.

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Declaration

The work presented in this dissertation was carried out at the Wellcome Trust Sanger Institute (Hinxton) and at the Department of Pathology (Tennis Court Road, Cambridge) between October 2007 and September 2011. This dissertation is the result of my own work – contributions from collaborations are clearly referenced and have been approved by the collaborators. No part of this dissertation has been or is being submitted for any qualification in any other university.

This thesis does not exceed the word limit established by the Biology Degree Committee.

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This thesis is dedicated to my parents.

Summary

Schistosomiasis is an endemic parasitic disease affecting approximately a quarter of a billion people worlwide, mainly in developing and under developed countries of Africa, Southeast Asia and Central and South America. The causative agent is a plathyhelminth worm of the genus *Schistosoma.* Chemotherapy with praziquantel is possible but does not protect from re-infection; moreover, reduced susceptibility to this drug have raised the issue of potential outbursts of drug resistance. In this context, researchers have a strong interest in finding alternative routes of chemotherapy and have also established programs for vaccine development. It is thought that the most vulnerable point in the parasites' life cycle is the early stages of life in the mammalian host. The infectious larvae, the cercariae, infect the host by penetrating through the skin where parasites transform into schistosomula. *In vivo*, skin transformation occurs within hours and this process can be reproduced *in vitro* by inducing a mechanical transformation. The parasite profile of gene expression across this vulnerable transition is not well understood. What is more, a transcriptome comparison between naturally transformed parasites and those forced to transform *in vitro* has not been investigated.

This thesis aims at filling in these gaps in our knowledge of schistosome biology by investigating gene expression changes that the early schistosomula undergo upon infection. In order to address this question, RNA-seq transcriptome sampling of cercariae, 3-hours old and 24-hours old schistosomula and adult worms was used. Because RNA-seq differential expression analyses heavily relies on genome annotation, the transcriptome data was first used to improve the gene annotation of the *Schistosoma mansoni* genome. Second, RNA-seq data generated from 24-hours old skin- and mechanically transformed schistosomula were compared. Finally, the patterns of gene expression that accompany the transformation of the parasites from the cercariae stage to the schistosomula during its first 24 hours of infection were studied. This time course study allowed the identification of known biological processes with improved resolution while other newer developmental changes are also reported and examined. The resolution achieved in this study has no precedent in any other parasitic helminth and contributes to our understanding of schistosome biology.

Abbreviations

ATP	Adenosine triphosphate
CDS	Coding Sequence
ConA	Concanavilin A
DEPC	Diethyl pyrocarbonate
DMEM	Dulbecco's Modified Eagle's Medium
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
EST	Expressed Sequence Tag
FCS	Foetal calf serum
GO	Gene Ontology
MT	Mechanically-transformed schistosomula
NADH	Nicotinamide adenine dinucleotide
Npp	Neuropeptide precursors
nt	Nucleotides
ORF	Open Reading Frame
PCR	Polymerase Chain Reaction
qPCR	Quantitative Polymerase Chain Reaction
RPKM	Reads per Kilobase per million of reads mapped
SL	Spliced leader
ST	Skin-transformed schistosomula
TCA	Tricarboxylic acid cycle
TBE	Tris Borate EDTA
UbCRBP	Ubiquinol-cytochrome C reductase binding protein
UTR	Untranlated Region
WTSI	Wellcome Trust Sanger Institute

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