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Transcriptional profiling of the oesophageal gland region of male worms of *Schistosoma mansoni*

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ABSTRACT

The intestinal tract of schistosomes opens at the mouth and leads into the foregut or oesophageal region that is lined with syncytium continuous with the apical cytoplasm of the tegument. The oesophagus is surrounded by a specialised gland, the oesophageal gland. This gland releases materials into the lumen of the oesophagus and the region is thought to initiate the lysis of erythrocytes and neutralisation of immune effectors of the host. The oesophageal region is present in the early invasive schistosomulum, a stage potentially targetable by anti-schistosome vaccines. We used a 44k oligonucleotide microarray to identify highly up-regulated genes in microdissected frozen sections of the oesophageal gland of male worms of *S. mansoni*. We show that 122 genes were up-regulated 2-fold or higher in the oesophageal gland compared with a whole male worm tissue control. The enriched genes included several associated with lipid metabolism and transmembrane transport as well as some micro-exon genes. Since the oesophageal gland is important in the initiation of digestion and the fact that it develops early after invasion of the mammalian host, further study of selected highly up-regulated functionally important genes in this tissue may reveal new anti-schistosome intervention targets for schistosomiasis control.

1. Introduction

The intravascular environment provides the adults of schistosome blood flukes with a constant source of amino acids, which are primarily derived through the digestion of erythrocytic haemoglobin. The digestive tract in schistosomes opens at the mouth which leads into the foregut or oesophageal region. Lined with a syncytial epithelial layer that is in cytoplasmic continuity with the tegument lining, the oesophageal region develops early during cercarial transformation [1]. This region and the primary absorptive intestinal region develop rapidly after penetration of the host to initiate haemoglobinolysis. As the oral infolding of the tegument, the luminal cytoplasm of the oesophageal region is supported by cytons that lie embedded in parenchymal regions of the head. Posteriorly, the oesophagus takes on a secretory role. The synthetic apparatus of this region is centred in a cellular mass, the oesophageal gland, which encircles the oesophagus. The nature of this glandular region was first described in the 1960s [2,3], and its morphology described in the subsequent decade [2–5] but since then, limited attention has been paid to its further characterisation. However, recent analysis confirm that this region contains secretions that help the initial lysis of ingested red blood cells by showing haemoglobin leakage from the ingested red blood cells at the posterior oesophagus [6] and secretions which may act to neutralise host immune effectors, thereby contributing to the parasite's survival [6,7].

Exploration of the transcriptional activity of the oesophageal gland is warranted, given its pivotal role in digestion. The acoelomate nature of the worm body has, to this point, limited study of the expression profiling and functional genomics of the oesophageal gland region as this part of the worm cannot be readily separated from other tissues using conventional methods. There is the additional problem of the very small size of the oesophageal gland, which in males is about 30 μm in diameter, and even less in females. A number of approaches have been employed to separate distinct

Abbreviations: OCT, optimal cutting temperature compound; QIMR-B, QIMR-Berghofer Medical Research Institute; GO, gene ontologies; cDNA, complementary DNA; VAL, venom allergen-like; MEG, micro-exon genes; SAPLIPs, saposin like proteins; WISH, whole mount *in situ* hybridisation.

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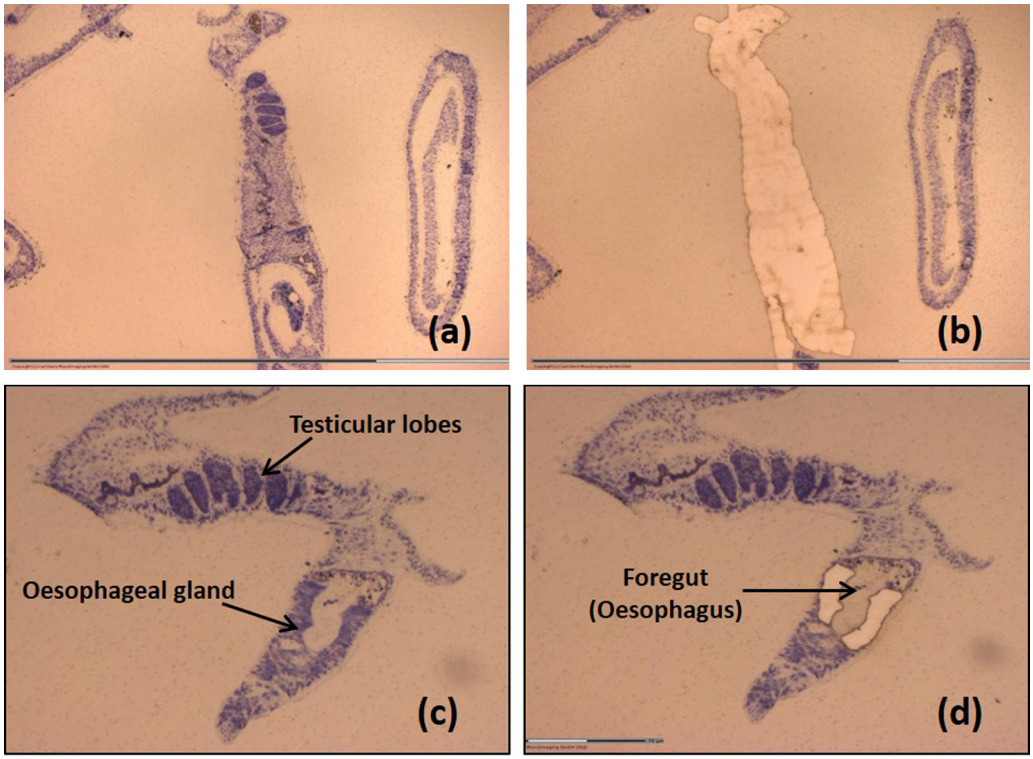

Fig. 1. Adult *S. mansoni* male worm sections used in laser microdissection of the oesophageal gland region compared to a micrograph of an unstained whole male worm anterior region. Worm sections were stained with 1% (w/v) toluidine blue. Image (a) shows the anterior part of a male worm before microdissection. Image (b) shows the space left after microdissecting the whole male worm section for the control sample. Images (c) and (d) show the male head region with oesophageal gland before microdissection and the space left after microdissection respectively (Scale bar = 75 μm).

tissues of schistosomes for transcriptional profiling. These include laser microdissection microscopy [8], a technique that has proven invaluable for isolating compact tissues, such as the gut lining and gonadal tissues, that can be stained, and used for subsequent analysis. More recently, an enzymatic digestion protocol has been developed for isolation of ovarian and other tissues [9].

In this paper, we have broadened the gene atlas of *Schistosoma mansoni* [8,10] by providing an analysis of the transcriptional activity of the oesophageal gland of male parasites. Due to the minute size of the gland in females, it has not been possible to microdissect those tissues.

2. Materials and methods

2.1. Sample preparation

The Puerto Rican strain of *S. mansoni* is maintained in ARC Swiss mice and *Biomphalaria glabrata* snails at QIMR-Berghofer Medical Research Institute (QIMR-B) from stock originating from the National Institute of Allergy and Infectious Diseases Schistosomiasis Resource Centre, Biomedical Research Institute (Rockville, Maryland, USA). *S. mansoni* is maintained at QIMR-B under permit from the Australian Department Agriculture, Fisheries and Forestry Biosecurity (DAFF). All animal work was approved by the Animal Ethics Committee of QIMR-B under Project P1289 of QIMR and the Animal Welfare Unit of The University of Queensland.

Adult worm pairs of *S. mansoni* were perfused from mice into perfusion buffer containing 145 mM sodium chloride and 58 mM sodium citrate. Approximately 40 male parasites were separated from females and transferred into Tissue-Tek optimal cutting temperature compound (OCT) (ProSciTech, Thuringowa, Australia) in a sterile plastic mould, which was frozen rapidly in dry ice and stored at −80 °C until use. Frozen sections (7 μm thick) were cut onto sterile glass slides that were coated with a 0.17 mm polyethylene naphthalate membrane (Carl Zeiss Microimaging GmbH, Bernried, Germany). Slides were stored at −80 °C until further use. Immediately prior to microdissection, the slides were thawed, washed in DEPC-treated RNase free water, stained with 1% toluidine blue (CHROMA, Stuttgart, Germany) to reveal the oesophageal gland and air dried in a sterile biohazard hood, just before microdissection as described [8,10,11].

2.2. Microdissection

Approximately 4×10^6 μm^2 of male *S. mansoni* oesophageal gland tissue was microdissected (Fig. 1) using a PALM MicroBeam Laser Catapult Microscope (Carl Zeiss Microimaging) and collected into 200 μl opaque adhesive Teflon-coated caps (Carl Zeiss Microimaging) located in the laser path, a few millimetres above the specimen stage as described [10]. For the control sample, an area of approximately 20×10^6 μm^2 was microdissected randomly from comparable regions of whole male worm tissue sections made exactly in the same manner as the test samples (Fig. 1).

2.3. Total RNA isolation and hybridisation

Total RNA was isolated from laser microdissected samples using RNAqueous-Micro kits (Ambion, Austin, TX, USA) using the manufacturer's protocol for LMM samples. The quality of RNA was assessed using a Bioanalyser RNA Pico Lab Chip (Agilent Technologies, Santa Clara, CA, USA) as described [10].

A 44k oligonucleotide microarray platform (Agilent Technologies) designed specifically for *S. mansoni* [10,11] was used for microarray analysis [8,10]. Two biological replicates were used for each sample. An Agilent low input Quickamp Labelling kit was used for microarray hybridisation. Twenty nanograms of total