Molecular & Biochemical Parasitology 196 (2014) 21–28

Contents lists available at ScienceDirect

Molecular & Biochemical Parasitology

Review

Gaining biological perspectives from schistosome genomes

Geoffrey N. Gobert*, Hong You, Donald P. McManus

Molecular Parasitology Laboratory, Infectious Diseases Division, QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia

ARTICLE INFO

Article history:
Received 1 May 2014
Received in revised form 14 July 2014
Accepted 17 July 2014
Available online 27 July 2014

Keywords:
Schistosoma
Helminth
RNAi
Systems biology

ABSTRACT

Characterization of the genomic basis underlying schistosome biology is an important strategy for the development of future treatments and interventions. Genomic sequence is now available for the three major clinically relevant schistosome species, *Schistosoma mansoni*, *S. japonicum* and *S. haematobium*, and this information represents an invaluable resource for the future control of human schistosomiasis. The identification of a biologically important, but distinct from the host, schistosome gene product is the ultimate goal for many research groups. While the initial elucidation of the genome of an organism is critical for most biological research, continued improvement or curation of the genome construction should be an ongoing priority. In this review we will discuss prominent recent findings utilizing a systems approach to schistosome biology, as well as the increased use of interference RNA (RNAi). Both of these research strategies are aiming to place parasite genes into a more meaningful biological perspective.

Contents

1. Introduction

Schistosome blood flukes infect approximately two hundred million people throughout 76 countries, with six hundred million at risk [1]. Adult schistosomes mate and travel into the venous habitat of the definitive mammalian host, where they produce eggs, potentially for decades. The deposition of schistosome eggs is the core pathological cause of chronic schistosomiasis [2]. The clinical symptoms associated with schistosomiasis range from fever, headache and lethargy to severe fibro-obstructive pathological changes. The disease often leads to portal hypertension, ascites and hepatosplenomegaly. Meta-analysis of the disease [3] indicates that, in terms of morbidity and mortality, the disease burden on individuals and communities due to schistosomiasis has increased worldwide, with DALY estimates (Disability-Adjusted Life Years) rising ~20% from 40-48/100,000 in the past 20 years. Current control involves targeted or mass drug administration with the heterocyclic pyrazino-isoquinoline praziquantel. Treatment relies solely on the anthelmintic praziquantel and, with increased usage, comes increased chances of resistant parasites developing [4].

2. Schistosome genomes

Complete genomic sequence is now available for the three major clinically relevant schistosome species, *Schistosoma mansoni*,

Abbreviations: Lgl, lethal giant larvae; SjColV, *Schistosoma japonicum* schistosomula collagen V; SGTP, *Schistosoma* glucose transporter protein; ds-RNA, double stranded RNA; sh-RNA, short hairpin RNA; si-RNA, short or long-interfering RNA.

* Corresponding author. Tel.: +61 7 3362 0406; fax: +61 7 3362 0104.
E-mail addresses: geoffG@qimr.edu.au, Geoffrey.Gobert@qimrberghofer.edu.au (G.N. Gobert).

http://dx.doi.org/10.1016/j.molbiopara.2014.07.007
0166-6851/

S. japonicum and *S. haematobium*, and this information represents an invaluable resource for the future control of human schistosomiasis [5–7].

A report described the use of FISH (fluorescence *in situ* hybridization) to physically map genomic scaffolds for *S. mansoni* to chromosomal spreads of the seven autosomes and the sex-determinant (ZW) chromosomes [8]. The results from this approach were extended to the *S. japonicum* genome by applying the scaffold from *S. japonicum* to the FISH validated *S. mansoni* scaffolds, using homologous synteny blocks (HSBs). HSBs represent large sections of a scaffold which share at least two markers between the two species with a conserved order, allowing assignment to a particular chromosome. Differences in HSBs between the two species were identified as "Evolutionary Breakpoint Regions" and were associated with sites where intra-chromosomal rearrangements can occur; with some of these sites subsequent identified in a study of two geographical strains of *S. japonicum* [9], supporting their *in silico* identification.

While the initial elucidation of the genome of an organism is critical for most biological research, continued improvement or curation of the genome construction should be an ongoing priority. An updated version of the *S. mansoni* genome has been released [10] three years after the original draft [7], with distinct improvement in the areas of chromosomal mapping and a reduction in the overall number of contigs and scaffolds. Of the 10,852 genes identified within the *S. mansoni* genome, many still do not have any useful annotation, although the majority (81%) have now been assembled onto a chromosome scaffold, representing a genetic map [10,11]. This provides a useful resource to allow the examination of broad chromosomal rearrangements that, for example, may have occurred between species or between geographical isolates. The latter was reported using comparative genomic hybridizations for *S. japonicum* endemic to mainland China and the Philippines but the study was limited and rudimentary due to the application of an incomplete microarray-based approach [9]. Finally the updated version of the *S. mansoni* genome also included extensive RNA-seq analysis to profile the transcriptional status of cercariae, 3 h schistosomules, 24 h schistosomules and adult parasites. While the four lifecycle stages examined represent key phases in schistosome development, it was unfortunate that mechanically transformed schistosomules were used while *in vivo* derived stages such as lung schistosomules were not included, especially as important transcriptional differences that have been reported in *S. japonicum* [12]. On the other hand, a recent study by Protasio and colleagues [13] compared gene transcription in skin-transformed and mechanically transformed schistosomula and found few differences in differential expression. Secondly, the RNA-seq analysis of the adult stage utilized a mixture of male and female parasites, thus excluding the identification of novel sex specific differential expression, which has been a focus of past research using relatively limited microarray technology [14–16]. It is, however, clear that improved fidelity of the *S. mansoni* genome does allow a much better approach for the use of many downstream applications including RNA-seq analysis to explore the transcriptional modulation of the parasite under changing conditions.

The last of the major schistosome species to have its genome sequenced was *S. haematobium* [5]. Overall characteristics of the *S. haematobium* genome were similar to those described for *S. japonicum* and *S. mansoni;* genome size was estimated at 385 Mb, 13,073 putative coding genes were identified, while gene size, GC content, exon numbers and repeat rates were comparable. Unsurprisingly, the genome of *S. haematobium* showed closer synteny or overall genomic structure with *S. mansoni* (89.4%) than *S. japonicum* (51.7%) [17]. The relative differences between the three species was also reflected in the intra-chromosomal rearrangements in *S. japonicum* which occurred four times more frequently than in *S. haematobium* or in the well characterized *S. mansoni* genome. Using the approach previously applied to *S. japonicum* [8], the *S. haematobium* genome was aligned to *S. mansoni* supercontigs (scaffolds) to allow subsequent mapping to specific chromosomes. Transcriptional analysis using RNA-seq allowed both the calculation of the number of gene-encoding sequences but also the exploration of the adult and egg stages of the parasite, with a focus specifically on important biological functions associated with the pathology caused within the definitive host. A defining features of *S. haematobium*, when compared with the other two major species, is that eggs from adult parasites lodge not in the host intestine but the in bladder. Subsequently the pathology results in urinary schistosomiasis and, most importantly, the squamous cell bladder carcinoma often associated with the chronic disease state [18]. Egg-enriched, female- enriched and male-enriched genes were identified in *S. haematobium* by RNA-seq and many correlated with previous studies on *S. mansoni* and *S. japonicum* using microarray platforms [19,20]. A finding related to the *S. haematobium*-egg induced pathology included the presence of IPSE a secreted glycoprotein which induces IgE (immunoglobulin E) production of IL-4 (interleukin 4), an observation originally proposed in *S. mansoni* to represent the immune-modulation of basophils [21]. This hypothesis has been further tested in a murine model involving the microinjection of *S. haematobium* eggs into the bladder which made resistant animals susceptible to bacterial infections [22], supporting the premise of active immune-modulation. Furthermore, in the same study, Hsieh and collegues [22] used ablation of the IL-4 receptor (IL-4Rα) to return the egg microinjected animal model to a bacterial resistant phenotype.

3. A systems approach to schistosome biology

Phylogenomics has been applied to schistosomes so as to place their proteomes into a broad evolutionary context with other parasitic and reference species as a novel approach to better understand their biology, particularly their interactions with their hosts [23]. Using this broad approach, insights into a wide range of features associated with host–parasite interactions were obtained with the primary aim of discovering new vaccine or drug targets. In the study, thirteen proteomes of key reference and parasitic species of evolutionary significance, including *Monosiga brevicollis*, *Ciona intestinalis*, *Nematostella vectensis*, *S. haematobium*, *S. mansoni*, *S. japonicum*, *Caenorhabditis elegans*, *Ascaris suum*, *Brugia malayi*, *Trichinella spiralis*, *Drosphila melanogaster*, *Tribolium castaneum and Homo sapiens*, were compared. From over 11000 *S. mansoni* proteins, almost 8000 phylogenetic trees were produced using an automated phylogenetic pipeline, representing approximately 70% coverage of the proteome of the parasite. It is important to note that, of the proteins assigned to phylogenetic trees, over one third had no previously assigned annotation. While relatively well known proteins such as tetraspanins and venom allergen-like proteins (VALs) were discussed in an evolutionary context, from the phylogenetic trees produced, the true value of this dataset is the greater coverage of proteins with improved annotation within the proteome. For the remaining proteins, which did not produce a usable phylogenetic tree, these may represent unique transcripts of the parasite's genome and most certainly are highly diverged from the model proteomes examined, and must wait for further study to elucidate their possible functional significance.

HelmCoP (Helminth Control and Prevention) is a useful recently developed online resource combining data on helminth genomics, proteomics and the limited available information on functional genomics into a central database [24]. While the bulk of the database focuses on nematodes, both *S. mansoni* and *S. japonicum*