

Integrating transcriptome, proteome and glycome analyses of *Schistosoma* biology

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Publication of the transcriptomes of *Schistosoma mansoni* and *Schistosoma japonicum*, in conjunction with the sequencing and assembly of their genomes, has generated a comprehensive picture of *Schistosoma* transcriptional and genomic diversity. Subsequently, researchers who study conjugal and developmental biology, tegumental composition and larval or egg, secretory and excretory products have used these data, in combination with the latest ‘-omics’ technologies, to extend large-scale screens of the schistosome transcriptome, proteome and glycome. In this article, we review these postgenomic investigations and contend that the generated datasets provide a plethora of novel drug, vaccine and immunomodulatory targets that might be useful for developing new antischistosome agents.

Schistosomiasis and its increased perceived morbidity

Reassessment of the disability-adjusted life years for individuals who are affected by schistosomiasis indicates that this disease is a more serious problem than was previously estimated [1]. In fact, it has been suggested that the current World Health Organization (WHO: <http://www.who.int>) approximation of 0.5% disability should be raised as high as 15% to give a more accurate reflection of disease burden in the 74 tropical and subtropical areas of the world that are affected by these parasitic trematodes [2]. Although this reflects a sobering reality as to the magnitude of the ‘hidden’ schistosomiasis pandemic, research priorities within the schistosome community are being openly and positively discussed, with the aim of creating a global agenda of directed laboratory- and field-based investigations to address some of the most important issues related to schistosomiasis today (<http://www.bio.net/biomail/listinfo/schisto>).

One pressing topic is the clear and urgent need to identify new and effective antischistosome chemotherapies or immunotherapies – current treatment programmes rely on the effectiveness of a single chemotherapy, praziquantel (PZQ). Despite the favourable toxicological properties of PZQ, and its efficiency at killing adult schistosomes and their eggs, this compound is not a ‘magic bullet’ [3–6] and, arguably, should not be considered the future

antischistosome panacea. Furthermore, the translation of successful results obtained from laboratory-based, experimental vaccine studies in animal models to preliminary Phase I trials in humans has been difficult to achieve for the antigens that were selected as potential immunoprophylactic targets [7]. The reasons for this difficulty include complications in protein production, different interpretations concerning vaccine-induced efficacy read-outs and selecting the best strategy for vaccinating infected populations. The search for new drug targets and effective vaccine candidates is, therefore, a priority in developing novel strategies to combat this major pathogen of humans [8].

Novel target identification through improvements in postgenomic screens

The recent publication of the *Schistosoma mansoni* and *Schistosoma japonicum* transcriptomes [9,10], and their accessibility through public databases (<http://verjo18.iq.usp.br/schisto/>, <http://www.genedb.org/genedb/smanson/index.jsp> and <http://schistosoma.chgc.sh.cn/>), has made the expressed genomic content of these two pathogenic trematodes an extremely valuable resource for the instigation and continuation of many global postgenomic initiatives (defined here as DNA microarray profiling, proteomics and glycomics). Continued refinement of the *Schistosoma* genomes (<http://www.genedb.org/genedb/smanson/index.jsp>; <http://lifecenter.sgst.cn/sj.do>) will also aid the success of such activities. Although large-scale gene expression studies [expressed sequence tag (EST) analyses], proteomic investigations (2D gel electrophoresis) and glycomic examinations (antibody reactivities and structural determinations) were performed on schistosome parasites during the pregenomics era (before publication of the *Schistosoma* transcriptomes), identification of biological associations for each gene product (direct or indirect) has been substantially accelerated by technological improvements in the way that current postgenomic screens are performed and analysed.

For example, using contact printing of prefabricated oligonucleotides or noncontact inkjet *in situ* printing of oligonucleotides [11], it is now possible to fabricate DNA microarrays at densities that are sufficient to represent the whole *Schistosoma* transcriptome (~14 000–20 000 transcripts). Improvements in target amplification methodology

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[12] enable DNA microarray hybridizations to be performed with as little as 50–500 ng of total parasite RNA [13]. In addition, the recent development and use of advanced Bayesian statistical modelling technology and spot-finding algorithms [e.g. Bluefuse (BlueGnome: <http://www.cambridgebluegnome.com/>)] have greatly improved the detection of low-hybridization signal intensities, have facilitated more-accurate discrimination of signal versus noise and, importantly, have led to a reduction in experimental variability across replicate hybridizations. With the maturation of large-scale proteomics and glycomics technologies, the patterns of parasite proteins and glycosylation can also be readily obtained. Modern time-of-flight, ion-trap or ion-cyclotron mass spectrometry (MS), powerful separation techniques [e.g. 2D gel electrophoresis and nanoliquid chromatography (LC)], well-developed experimental strategies for (differential) proteomic and glycomic screenings, appropriate robotics and public sequence databases have greatly accelerated these discoveries at a previously inconceivable rate [14–17]. Protein identifications can now be routinely performed from femtomole amounts of material in a gel plug or in solution, and LC–tandem MS (MS–MS) approaches, combined with the latest data-mining software (dedicated algorithms that can link MS fingerprints or MS–MS fragmentation spectra to nucleotide or protein sequence databases), enable the classification of thousands of proteins in a mixture. Moreover, using MS-based approaches, schistosome glycans (highly relevant in schistosome biology and immunology [18–20]) can now also be detected at a level of sensitivity and complexity necessary to merge global glycomic studies with large-scale proteomic and/or transcriptomic investigations. Importantly, it is the technological advancement in glycosylation analysis and not the deciphering of genomic sequence information itself that, unlike for proteomics [in which peptides can be directly matched to open reading frames (ORFs) derived from genomic sequence], has fuelled this schistosome glycomic revolution.

Together, these technological improvements are greatly increasing the speed with which schistosome gene products are being linked to well-studied and novel areas of parasite–host biology. In this article, we review how postgenomic studies have impacted major areas of schistosome biology, specifically focusing on the male–female dichotomy and the development of cercariae, schistosomula and eggs. We believe that the collective use of these complementary approaches has provided the community with a repository of necessary information that underpins present and future hypothesis-directed drug discovery and vaccine-related research undertakings.

Postgenomic investigations of dioecy in the adult schistosome

Schistosomes have evolved a dioecious (separate male and female individuals) lifestyle that facilitates distinct functionality among adults and maximizes reproductive success and fitness through conjugal biology. The most important outcome of conjugal biology is the production of nonoperculate (absence of a preformed operculum, or pore, in the egg shell) eggs, which is simultaneously linked to life cycle transmission and most host immunopathologies. Therefore, large-scale postgenomic screens of the

dioecious state might identify gene products that are differentially associated with each sex and focus attention on those products, pathways or structures that have biological roles in the formation of sexually mature schistosome interactions. Furthermore, these investigations could also reveal clues that are related to the biology of egg production, the identity of molecules that influence immunopathology or immunomodulation and the description of novel chemotherapeutic targets.

Transcriptomics (DNA microarray profiling)

Towards this end, DNA microarray investigations that examine transcriptional differences between sexually mature adult male and sexually mature adult female schistosomes have been performed [21–25] (Figure 1). These studies, using cDNA [21] and oligonucleotide DNA microarrays (either 50-mer [23] or 60-mer targets [25]), have profiled RNA from both *S. mansoni* (the Puerto Rican strain [21,23] and an unknown strain [24]) and *S. japonicum* (the Anhui and Zhejiang Chinese strains [22] and the Chinese and Philippine strain [25]). In all but one study [25], these investigations demonstrated that adult female schistosomes differentially express larger numbers of diversely classified transcripts than do adult male schistosomes. Although the exact numbers of differentially expressed, gender-associated transcripts were unique in each study, substantial correlation was found among the gene products displaying substantial sequence database annotation (most of the differentially expressed transcripts in these studies did not have database homologues). The majority of these studies confirm that the female-associated expression of many known eggshell-related transcripts, in addition to other previously characterized gene products, is transcribed differentially in the adult female (e.g. female-specific 800 protein, ferritin-1 and ORF-reading frame 2). Correlations were also found among studies regarding the female-biased expression of genes that share sequence similarity to histidine-rich proteins, vesicular-trafficking components, DNA metabolism and nucleotide-salvage gene products, aspartic proteinases, tyrosinases, cell-cycle-associated kinases, antioxidant enzymes, transaminases, ribosomal proteins and neutral-lipid-synthesizing enzymes. These gene products, according to proposed gene ontology nomenclature (<http://www.geneontology.org/>), probably have a role in the prolific production of vitelline cells (e.g. vesicular trafficking, DNA metabolism, nucleotide salvage and cell-cycle-associated kinases), the hardening of eggshells (tyrosinases), the production of eggs (tyrosinases and ribosomal proteins), the digestion of host red blood cells (aspartic proteinases and transaminases) and the elimination and storage of toxic metabolites (histidine-rich proteins, antioxidant and neutral-lipid-synthesizing enzymes).

Although incomplete, confirmatory evidence from complementary postgenomic approaches [26] and ongoing functional investigations [27] support the pursuit of some of these products as novel chemotherapeutic or immunoprophylactic targets because of complete conservation between transcriptional results and proteomic or enzymatic observations (some potential targets include extracellular superoxide dismutase and aspartate amino

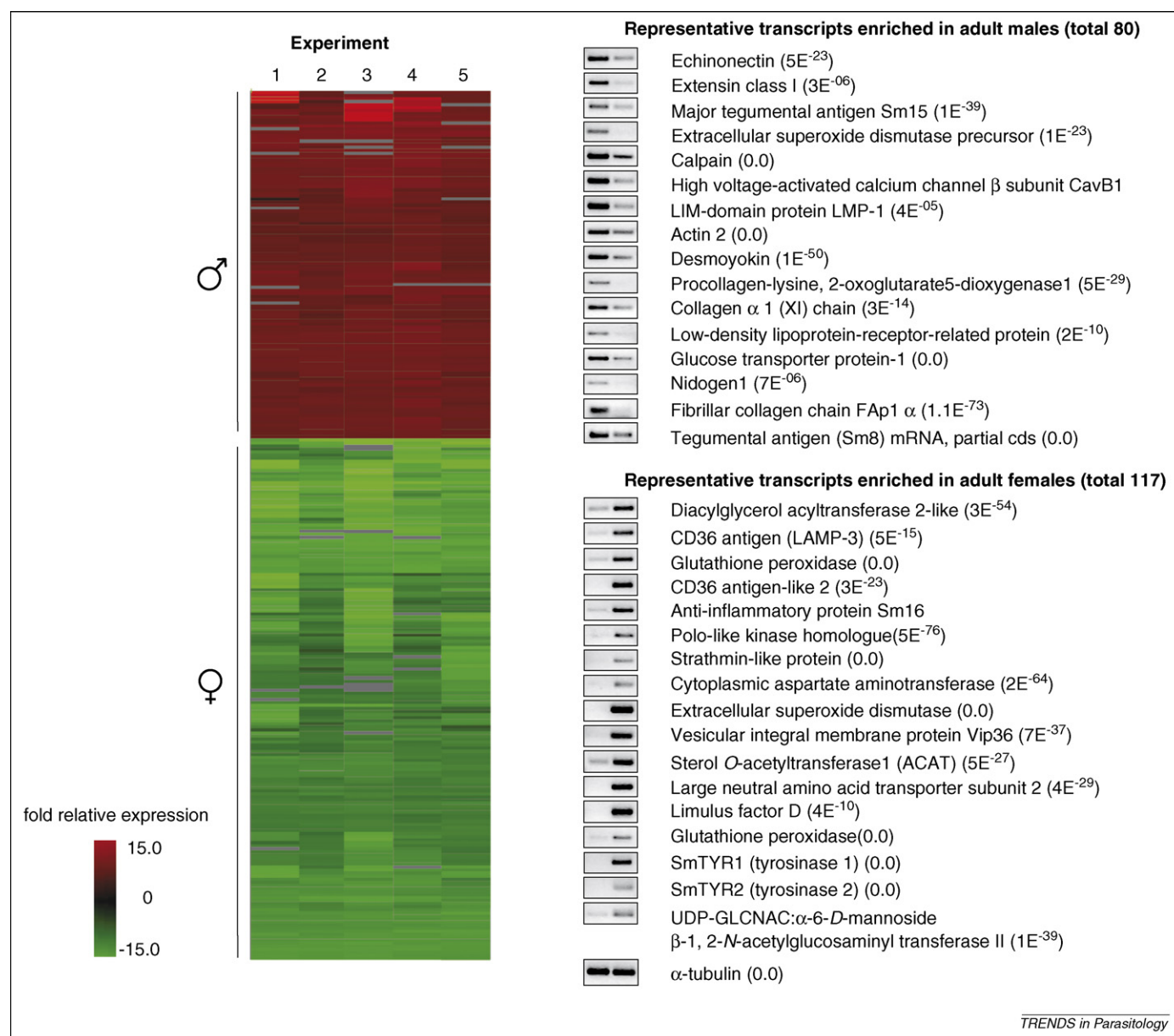


Figure 1. *Schistosoma mansoni* microarray gene expression profiles. *S. mansoni* oligonucleotide microarrays reproducibly detect gender-enriched gene expression profiles in sexually mature male and female parasites. Single-linkage hierarchical clustering (using euclidean distance correlation matrices) of all gender-enriched transcripts (197 total) that were identified in a recent study by Fitzpatrick *et al.* [23]. Representative gender-enriched transcripts were verified by reverse-transcription PCR (RT-PCR) and compared with α -tubulin, and are included next to the clustergram along with their unique identifier (Contig identification, accession number or name) and corresponding BLASTx (http://www.incogen.com/public_documents/vibe/details/NcbiBlastx.html) value. Brighter shades of red depict greater expression in adult male parasites, brighter shades of green represent greater expression in adult female parasites, and grey denotes that data are absent for that particular replicate experiment. DNA microarray hybridizations were performed five times from five independently collected batches of adult male and female parasites (each experiment is represented by a number at the top of the clustergram).

transferase) (Figure 2). The extent of such conservation (dependent upon schistosome post-transcriptional and translational regulation) and its relative importance during female sexual biology will be deduced only through systematic and thorough studies of each molecule. Towards this end, several database resources are currently available to shortlist candidate gene products (including those listed in Table 1).

In contrast to the differentially expressed female products, male-biased transcripts are less numerous and diverse, and seem to be associated with muscle function, structural support, calcium and glucose homeostasis, and extracellular and intracellular architecture. Although

these transcriptional findings lend support to Basch's theory of schistosome dichotomy (i.e. adult male worms provide solely intravascular support for the specialized egg-laying adult female [28]), there are more-complex interactions that occur between adult male and adult female parasites. This was demonstrated in a recent study that examined gene expression profiles of schistosomes from mixed-sex versus single-sex murine infections [29]. Further studies to investigate the mechanism of contact-dependent transcriptional regulation in adult schistosomes should provide details about the molecules involved, subsets of which might be suitable targets for novel chemotherapies to block conjugal biology.

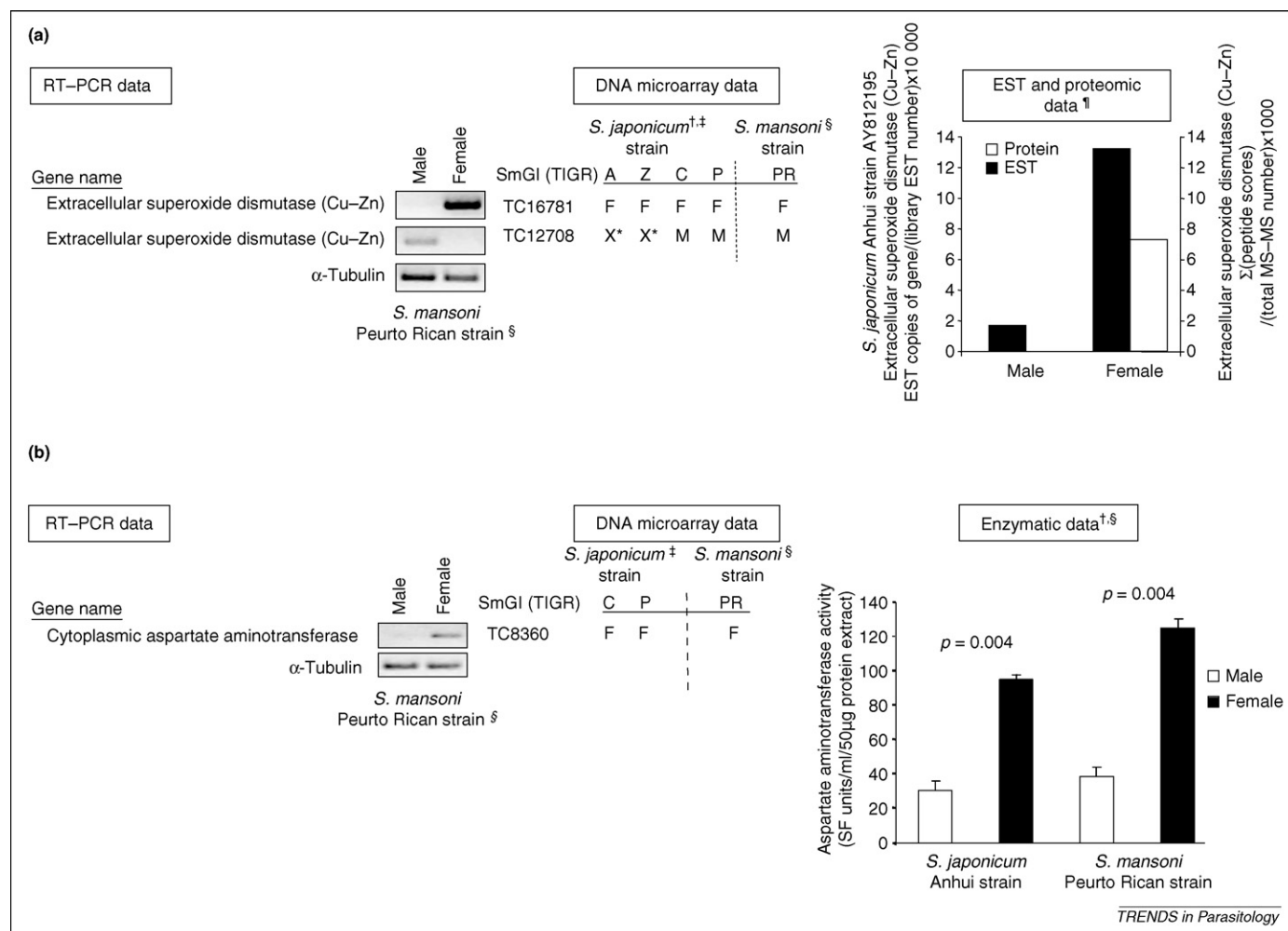


Figure 2. Postgenomic analyses of schistosome gene products. Transcriptome and proteome postgenomic analyses of schistosome gene products can provide complementary information for the assignment of gender associations across species and strain. **(a)** Amalgamation of three different methods (RT-PCR, DNA microarray profiling and a combined EST and proteomic survey) to assign gender association for *Schistosoma* extracellular superoxide dismutase (SOD) (Cu-Zn) homologues (SmGI = *Schistosoma mansoni* gene index numbers TC16781 and TC12708). **(b)** Amalgamation of three different methods (RT-PCR, DNA microarray profiling and enzymatic analysis) to assign gender association for *Schistosoma* cytoplasmic aspartate aminotransferase (SmGI = *S. mansoni* gene index number TC8360). *S. mansoni* RT-PCR and DNA microarray data originate from Ref. [23] and are indicated by '§'. *S. japonicum* DNA microarray and EST and proteomic data are adapted from Refs [22,25,26] and are indicated by '†' (Ref. [22]), '‡' (Ref. [25]) or '¶' (Ref. [26]). *Schistosoma* strains are indicated as follows: A, Anhui; C, Chinese; P, Philippine; PR, Puerto Rico; Z, Zhejiang. Female-biased gene expression (F) and male-biased gene expression (M) during DNA microarray profiling of either *S. japonicum* or *S. mansoni* parasite material are shown. X* indicates that gene product was not present in the profiled DNA microarray and, therefore, gene expression results could not be obtained. Enzymatic data originate from schistosome samples collected in Refs [22,23] using a commercially available enzyme assay kit (GO-Transaminase, Sigma-Aldrich: <http://www.sigmaaldrich.com>).

Table 1. DNA microarray studies of schistosome biology: accessibility of MIAME-compliant primary gene expression data^a

DNA microarray specifics			DNA microarray database reference numbers			Refs
DNA microarray type ^b	Species	Number of elements	Database ^c	Array reference	Experiment reference	
Oligonucleotide (50-mers), academic	<i>Schistosoma mansoni</i>	16 320	EBI, Array Express	A-MEXP-134	E-MEXP-223	[23]
cDNA, academic	<i>S. mansoni</i>	13 056	EBI, Array Express	A-SGRP-2	E-SGRP-2	[53]
Oligonucleotide (60-mers), commercial	<i>S. mansoni</i> and <i>Schistosoma japonicum</i>	22 575	NCBI, Gene Expression Omnibus	GPL3821	GSE5235	[11]
Oligonucleotide (60-mers), commercial	<i>S. mansoni</i> and <i>S. japonicum</i>	22 575	NCBI, Gene Expression Omnibus	GPL3821	GSE5234	[25]
Oligonucleotide (60-mers), commercial	<i>S. mansoni</i> and <i>S. japonicum</i>	22 575	NCBI, Gene Expression Omnibus	GPL3821	GSE5191	[13]
Oligonucleotide (50-mers), academic	<i>S. mansoni</i>	16 320	EBI, Array Express	A-MEXP-134	E-MEXP-474	[29]
cDNA, academic	<i>S. mansoni</i>	9216	NCBI, Gene Expression Omnibus	GPL3929	GSE5233	[24]

^aAbbreviation: MIAME, minimal information about a microarray experiment.

^bAcademic or commercially manufactured microarrays.

^cEBI, Array express: European Bioinformatics Institute (<http://www.ebi.ac.uk/arrayexpress-old/>). NCBI, Gene expression omnibus: National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/projects/geo/>).

Proteomics

Proteomic analyses of schistosome dioecy have been performed and complement the transcriptomic studies described [26,30]. An interesting general notion from one of these studies is that gender-biased protein expression is more substantial than stage-enriched expression [26]. In this combined proteome and EST analysis, 1375 proteins were identified in adult *S. japonicum* worms: of these, 491, 574 and 723 were identified from females, males and mixed adults, respectively. Furthermore, 60% of proteins that were identified in female and male protein extracts were also represented by an EST in the complementary-generated transcriptomic (EST) dataset. However, comparison and integration of transcriptomic and proteomic data, as performed by Liu *et al.* [26], show that, except for a small number of examples (Figure 2), the two datasets are complementary rather than comparable. Correlation between the presence and the quantity of a transcript and its protein end-product is dependent on a range of regulatory and metabolic events. However, another major reason for the observed discrepancy [26] is almost certainly of a technical or experimental nature that is intimately related to the difficulties in extracting transcriptome and proteome information derived from multiple and highly complex tissues of these metazoan parasites. Despite the fact that a large number of proteins can be identified as constituents of highly complex mixtures using LC-MS-MS approaches, the coverage of a proteome by MS-based methods is still not comprehensive. This is because of the large dynamic range covered by most of the complex protein extracts, the biophysical properties of proteins, which render some more difficult to detect than others, and post-translational modification, which might further complicate protein identification. In addition, incomplete sequence information (EST-derived databases) limits the success of using peptide-mass fingerprinting or MS-MS fragmentation search strategies.

Glycomics

Long before the initiation of large-scale transcriptomic and proteomic screens, the stage-specific expression of schistosomal protein- and lipid-linked glycans or glycan elements was reported [31–36]. Developmental regulation is a feature of schistosome glycosylation that cannot be overlooked in view of the immunobiological roles that are attributed to schistosome glycans during host–parasite interactions [18,19,37]. Further study of the dioecious transcriptomic and proteomic data indicated a group of female-biased gene products (e.g. dolichyl-diphosphooligosaccharide-protein glycotransferase, UDP-*N*-acetylglucosamine-dolichyl-phosphate, *N*-acetylglucosaminophosphotransferase, dolichyl-diphosphooligosaccharide-protein glycosyltransferase, β -1,4-galactosyltransferase, UDP-Gal: β GlcNAc β 1,3-galactosyltransferase III and UDP-GlcNAc α -6-D-mannoside β -1,2-*N*-acetylglucosaminyltransferase II [23,25,30]), all of which are enzymatic components of the glycosylation machinery. Differential expression of these transcripts and proteins indicates the occurrence of gender-specific protein glycosylation in the adult schistosome.

To address this systematically, a comparative glycomics study that employed MS-based profiling of *N*-glycan pools

released from *S. mansoni* adult male and female worms was performed [38]. Results demonstrated that females produce a larger proportion of highly branched structures, whereas males express a larger number of truncated glycans. Moreover, the female glycan pool contained a large proportion of β 1-4-linked galactose-terminating glycans [e.g. *N*-acetylglucosamine (LacNAc) and Lewis X (LeX) (Table 2)], whereas, in male-derived glycans, these termini were more often formed by β 1-4-linked *N*-acetylgalactosamine [e.g. LacdiNAc (LDN) and fucosylated LacdiNAc (LDN-F)]. Information about the enzymatic activity and product specificity of each of the gender-associated ‘glycogene’ products to correlate individual gene expression with the expression of their glycan products are not yet available. However, it is clear that predictions of gender-specific glycosylation (obtained from both transcriptomic and proteomic studies) can be corroborated by global glycomics profiling. This conclusion is further substantiated by immunofluorescence microscopy of worm sections using anti-LeX and anti-LDN-F monoclonal antibodies (mAbs), which indicated that the differential expression of these glycan epitopes is especially evident in the tegument (e.g. LeX is in the female tegument only and LDN-F is in the male tegument of the gynecophoral canal [38]). LDN, LDN-F and LeX are among the many different glycan elements to which antibody responses are raised in infected hosts during different immunological settings [39–42]. Here, they invoke cellular responses through the interaction with lectins and pattern recognition receptors [43–45]. Their (differential) location in the tegument indicates a potentially important role in the interaction with the host, in addition to between the male and the female worms.

Tegumental proteomics and glycomics

Differences in the tegumental proteome of male and female *S. japonicum* worms were also observed [26], although it was indicated that these differences might reflect experimental heterogeneity, rather than biologically important variation. It would be interesting to verify whether the differential expression of immunogenic glycan elements occurs on the same proteins in male and female worms or whether the underlying proteins, which carry these glycans, are gender associated themselves. Recently, the tegumental proteome of mixed-sex adult *S. mansoni* worms was studied [46–48]. Several proteins that are associated with well-known specific functions of the tegument (e.g. metabolite transport, signaling and membrane structure) were reproducibly found, along with some proteins that demonstrate functions that were previously unknown and are possibly unique in tegumental biology (e.g. male–female interactions and host–parasite interplay). The glycosyl modifications of these tegumental proteins are currently unknown. A more recent study of the *Schistosoma bovis* tegumental proteome suggested the abundant presence of proteins that are involved in cytosol energy metabolism and cytoskeleton architecture [49] and are similar to those proteins found in *S. mansoni* tegumental extracts [46,47]. In addition, a lectin- and antibody-based profiling study indicated that many tegumental proteins of *S. bovis* adult worms carry fucosylated

Table 2. Typical glycans and glycan elements expressed by schistosomes^a

Abbreviation or name	Sequence or structure
LDN	GalNAcβ1 – 4GlcNAcβ1 – R ^b
LDN-F	GalNAcβ1 – 4GlcNAcβ1 – R Fuca1 – 3
F-LDN	Fuca1 – 3GalNAcβ1 – 4GlcNAcβ1 – R
DF	Fuca1 – 2Fuca1 – R
DF-LDN-DF ^c	Fuca1 – 2Fuca1 – 3GalNAcβ1 – 4GlcNAcβ1 – R Fuca1 – 2Fuca1 – 3
LN	Galβ1 – 4GlcNAcβ1 – R
LeX	Galβ1 – 4GlcNAcβ1 – R Fuca1 – 3
Poly-LeX	[–3Galβ1 – 4GlcNAcβ1] _n – R Fuca1 – 3
Pseudo-LeY	Fuca1 – 3Galβ1 – 4GlcNAcβ1 – R Fuca1 – 3
CAA	[–6GalNAcβ1] _n – R GlcAβ1 – 3
Core-α6-Fuc^d	Manα1 – 3 Manα1 – 6 Fuca1 – 6 Manβ1 – 4GlcNAcβ1 – 4GlcNAcβ1 – Asn
Core-α3-Fuc^d	Xylβ1 – 2 Fuca1 – 3
Core-β2-Xyl^d	Man α1 – 6 Manα1 – 6 Man α1 – 3 Manβ1 – 4GlcNAcβ1 – 4GlcNAcβ1 – Asn
Mannosidic ^e	Man α1 – 2Man α1 – 3

^aSchistosomes express numerous glycans and glycan elements. This table lists only a representative summary that is based on the glycomic analyses of various schistosome preparations performed so far. Most elements can occur on protein-bound glycans (e.g. *N*-glycans and *O*-glycans) and lipid-bound glycans. Glycans can be found in multiple developmental stages of *Schistosoma mansoni* and *Schistosoma japonicum*. For more information on stage-specific expression, localization and references, see Table 3. Abbreviations: Asn, asparagine; CAA, circulation anodic antigen; Fuc, fucose; Gal, galactose; GalNAc, *N*-acetylgalactosamine; GlcA, glucuronic acid; GlcNAc, *N*-acetylglucosamine; Man, mannose; Xyl, xylose.

^bR, reducing end part of carbohydrate chain.

^cMultifucosylated LDN elements also occur in other variants, with *O*-2 fucoses linearly attached to each GalNAc and *O*-3 fucoses attached to each GlcNAc, which can also occur as a repeating fucosylated -4GlcNAcβ1- element.

^dα3-linked Fuc, α6-linked Fuc and β2-linked Xyl are *N*-glycan core modifications that might be expressed simultaneously or separately, depending on stage and schistosome species.

^eOligo- or paucimannosidic *N*-glycans, the number of mannose residues in the branches can vary.

glycan elements, including LeX [50]. It has yet to be determined whether these tegumental proteins or glycosyl modifications are gender associated.

Additional studies of conjugal biology will further unravel the complexities of this process; however, these combined postgenomic investigations have already contributed much to the understanding of the molecules associated with dioecy. Amalgamating results from these complementary investigations should lead to a concise set of gene products that is available for studies related to vaccine development, immunomodulation, chemotherapy and immunopathology.

Postgenomic investigations of cercariae, schistosomula and egg biology

Host invasion is mediated by the free-living miracidial or cercarial life stages, which are infective to the intermediate snail host or the definitive vertebrate host, respectively. Therefore, some of the products that are expressed and released by cercariae or miracidia are directly responsible for successful host infection, immunomodulation and immunoevasion. Likewise, some molecules that are expressed and released by tissue-entrapped eggs are responsible for a T helper (Th) cell type 2 polarized immune response and granuloma formation in infected definitive hosts

[51]. To date, two DNA microarray investigations have examined gene expression (directly and indirectly) in these specific life stages [52,53]. In one study, a direct comparison of gene expression profiles from aquatic miracidia and the intrasnailed sporocyst was performed [52]. Vermeire *et al.* produced an informative database of transcripts that are associated with schistosome–snail interactions, the transition from a free-living lifestyle to that of a parasitic one and the induction of asexual reproductive processes [52]. In an unrelated investigation, a compilation of genes that are highly expressed in cercariae and eggs was indirectly inferred by comparison with other profiled life stages by a common reference (i.e. an RNA pool composed of an equal amount of all parasite samples used in the study) [53]. Unfortunately, Dillon *et al.* were focused solely on identifying transcripts that are differentially expressed in *in vitro* cultured schistosomula and, therefore, examination of cercarial or egg life-stage transcripts was not thoroughly explored [53].

Subsequent detailed proteomic investigations to identify the excretory–secretory (ES) components that are released during the transition from free-living *S. mansoni* cercariae to cultured schistosomula have provided abundant lists of proteins that are putatively linked to

invasion of the definitive host [54,55]. This is in contrast to studies that profiled only the complete soluble cercarial proteome [26,56]. Cercarial transformations were induced by two different procedures in these articles (lipid-induced and mechanical transformation), which led to the identification of different sets of ES components. These studies indicated that the transformation process induces the release of many acetabular and head gland contents [54,55]. Ensuing MS analyses of these products resulted in the identification of proteases that are involved in skin penetration and proteins that are associated with putative immunomodulatory activity [55,57]. The larval transformation process also probably results in major changes of molecules that are exposed at the surface. *S. mansoni* cercariae express numerous immunogenic glycan elements (e.g. LeX on N- and O-glycans and glycolipids, N-glycan core xylosylation or fucosylation, multifucosylated termini on O-glycans and pseudo-Lewis Y (LeY) glycolipids [31,34,58,59]), and interesting observations have been made regarding the localization of these glycans (Table 3). For example, although the whole cercarial surface is covered with multifucosylated LDN elements, LDN and LeX surface expression is restricted to the oral sucker [42,60]. However, after schistosomula transformation,

Table 3. Pattern of typical immunogenic glycans and glycan elements expressed by different *Schistosoma mansoni* life stages^a

Developmental stage	Expressed element ^b	Carrier ^c	Surface expression	ES expression
Cercariae	LDN	P	Oral sucker	ND ^d
	F-LDN	P, L	ND	ND
	LDN-F	P	Partial	ND
	DF-LDN-DF and variants	P, L	General	ND
	LeX	P, L	Oral sucker	ND
	Pseudo-LeY	L	ND	ND
	Core- α 6-Fuc	P	ND	ND
Schistosomula three hours	Core- β 2-Xyl	P	ND	ND
	LDN	P	General	ND
	LDN-F	P	General	ND
	LeX	P	General	ND
Adult worms	LDN ^e	P	Tegument	ND
	F-LDN	L	No	ND
	LDN-F ^e	P, L	Tegument (male)	ND
	DF-LDN-DF and variants	P, L	ND	ND
	LeX	P, L	Tegument (female)	ND
	Poly-LeX	P	Gut	Yes, CCA ^f
	CAA	P	Gut	Yes, CAA ^f
	Mannosidic	P	ND	ND
	LDN	P, L	Shell	Yes ^g
	F-LDN	P, L	Shell	Yes
Egg	LDN-F	P, L	Shell	Yes
	DF-LDN-DF and variants	P, L	Shell	Yes
	LeX	P	Shell	Yes
	Core- α 3-Fuc	P	ND	Yes
	Core- α 6-Fuc	P	ND	Yes
	Core- β 2-Xyl	P	ND	ND
	Mannosidic	P	ND	ND

^aThis compilation of *S. mansoni* glycomics data is based on results presented in Refs [31–34,38,39,42,58–61,65,66,68,69]. Glycomics profiling has been performed for other *Schistosoma* species, including *Schistosoma japonicum* [31–33] and *Schistosoma bovis* [50]. Differences compared with *S. mansoni* occur (e.g. core modifications) but many elements are not species specific. Expression of LDN, LDN-F and LeX has also been profiled in intramolluscan stages of *S. mansoni* [35].

^bOnly glycan elements that have been implicated in host immune responses have been included. Absence of an element from this column does not necessarily mean that it is not present in this stage, just that it has not been found (or looked for) so far. See Table 2 for sequences of the glycan elements.

^cP, present as a protein conjugate(s); L, present as a lipid conjugate. In the case of a protein conjugate, terminal elements might be present on N-glycans, O-glycans or both. Core modifications and mannosidic antennae are always part of N-glycans. The presence of LDN on adult worm glycolipids has not been tested. LeX has been found on egg glycolipids by MS [66] but is not easily detected by blotting [61].

^dND, no data available.

^eAlso occurs as linear repeats, mainly in male worms [38].

^fCAA, part of the gut-associated circulating anodic antigen; CCA, part of the gut-associated circulating cathodic antigen.

^gBased mainly on immunofluorescence microscopy of eggs in host tissue sections. LeX, LDN, LDN-F, core- α 3-Fuc and core- α 6-Fuc have been detected on N-glycans of the ES glycoprotein IPSE α 1 [68].

LDN, LDN-F and LeX glycan elements common to the adult worms appear on the whole schistosomulum surface [42]. Furthermore, the multifucosylated glycan elements and modifications of the *N*-glycan core that are common to cercariae and eggs seem to be expressed at much lower levels in schistosomula and adult worms [31,60,61]. Detailed glycomic profiling studies have yet to be performed on cercarial and schistosomula ES products.

The initial study of the soluble *S. mansoni* egg proteome, compared with larval and adult proteomes, did not indicate much change in expression of the ~40 major proteins during the life stage development [56]. In the more extensive *S. japonicum* study [26], the eggshell and miracidia proteomes were separately analysed and hundreds of proteins in each sample were identified. The finding of 918 miracidial proteins, of which only 412 were detected in intact eggs, would indicate that the miracidia undergo substantial changes during or after hatching. These changes are, in part, reflected by the abundance of particular motor and receptor proteins that were uniquely identified in the miracidia proteome. Of the 520 proteins found in eggshell preparations, only 258 were also found in the intact egg. Supporting evidence is needed to substantiate which differences are truly related to egg maturation or to structural organization and which are of experimental origin. Global proteomic and glycomic studies of egg ES products have yet to be reported but are being performed. Along with global glycomic analyses of cercarial or schistosomula ES products, these complementary investigations will advance knowledge of schistosome and host immunobiological relationships.

There is a strong interest in egg glycosylation because of the crucial role that is attributed to egg glycans such as LeX, LDN and fucosylated LDN derivatives in the induction of Th2 responses [62] and granuloma formation within the infected host [63,64]. Western blot studies of *S. mansoni* protein extracts from cercaria, adult worms and eggs, using a set of anticarbohydrate mAbs, demonstrated that fucosylated glycan elements are more abundant on egg glycoproteins compared with the other life stages analysed [61]. These observations correlate with the developmental activity of fucosyltransferases. In eggs, these fucosyltransferases are present at ~50 times the levels seen in other life stages [36]. Detailed MS-based structural studies of *S. mansoni* and *S. japonicum* egg protein glycosylation demonstrated that eggs produce highly complex *N*- and *O*-glycans [32,65], with terminal immunogenic elements such as LeX, LDN-F and the polyfucosylated LDN (DF-LDN-DF) variants also occurring on egg glycosphingolipids [32,66] and other developmental stages [31,34,58]. In *S. mansoni* eggs, core- α 6-fucosylated and core- β 2-xylosylated *N*-glycans also occur [33]. These glycans, which possibly carry additional immunogenic antennae elements, are strong inducers of Th2 responses in mice [67]. Interestingly, although transcriptome and proteome studies of female adult worms have indicated a high expression level of components in the egg (shell), these xylosylated or fucosylated egg *N*-glycans are not detected in the female glycome [38]. Limited information is available about the specific location of egg glycans in terms of both the ultrastructure of the egg and the protein-specific expression. Fluorescence

microscopy with characterized anticarbohydrate mAbs demonstrated that LDN, LeX, LDN-F and difucosylated LDN (LDN-DF) elements occur as constituents of the egg-shell, the miracidium and secreted glycoproteins [60]. The individual glycosylation analysis of one major secreted egg glycoprotein, IPSE/ α_1 [68], confirmed the presence of LeX, LDN and LDN-F termini on its glycans. Recently, it has also been shown that, in addition to glycoprotein and glycolipid secretions, *S. mansoni* eggs release unconjugated, multifucosylated oligosaccharides. These are released from eggs in amounts that are detectable in the urine of the infected host [69] but, so far, it is unclear whether these free glycans have implications for the biology of the egg, the miracidium or the host in the same way as do protein- or lipid-linked glycans.

Further postgenomic investigations of larval and egg biology promise to shed light on some of the most fascinating areas of schistosome–host interactions, which will undoubtedly impact the interrelated areas of parasitology, immunology and vaccinology.

Concluding remarks

Although postgenomic studies of schistosome biology are still in their infancy, continued integration of approaches can and will lead to unprecedented rates of information discovery. The potential of these discovery-driven investigations seems unlimited but should be tempered by the need to prioritize rational hypotheses for the improvement of human and animal health. Careful examination of existing postgenomic datasets (Table 1 and supplementary files provided by authors of other postgenomic studies [11,21–25,52,53]) will complement this endeavour by providing a much-needed repository of schistosome gene products that are implicated in crucial parasite biological functions or host–parasite interactions. Further hypothesis-directed biological and functional studies will provide detailed information related to the localization, developmental expression, immunomodulatory capacity and immunoprophylactic or chemotherapeutic potential of selected gene products. These investigations, coupled with technological advances in defining subproteomes, determining which glycan structures are on which proteins, describing the (glyco-) lipidome [70] and deciphering the immunome [71], will make a substantial impact on current and future drug discovery and vaccine-related research.

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