REVIEW ARTICLE

New Research Tools for Urogenital Schistosomiasis

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Approximately 200 000 000 people have schistosomiasis (schistosome infection). Among the schistosomes, *Schistosoma haematobium* is responsible for the most infections, which are present in 110 million people globally, mostly in sub-Saharan Africa. This pathogen causes an astonishing breadth of sequelae: hematuria, anemia, dysuria, stunting, uremia, bladder cancer, urosepsis, and human immunodeficiency virus coinfection. Refined estimates of the impact of schistosomiasis on quality of life suggest that it rivals malaria. Despite *S. haematobium's* importance, relevant research has lagged. Here, we review advances that will deepen knowledge of *S. haematobium*. Three sets of breakthroughs will accelerate discoveries in the pathogenesis of urogenital schistosomiasis (UGS): (1) comparative genomics, (2) the development of functional genomic tools, and (3) the use of animal models to explore *S. haematobium*–host interactions. Comparative genomics for *S. haematobium* is feasible, given the sequencing of multiple schistosome genomes. Features of the *S. haematobium* genome that are conserved among platyhelminth species and others that are unique to *S. haematobium* may provide novel diagnostic and drug targets for UGS. Although there are technical hurdles, the integrated use of these approaches can elucidate host-pathogen interactions during this infection and can inform the development of techniques for investigating schistosomes in their human and snail hosts and the development of therapeutics and vaccines for the control of UGS.

Keywords. Schistosomiasis; Schistosoma; *Schistosoma haematobium*; bladder; genomics; urogenital schistosomiasis.

Schistosomes are the etiological agents of human schistosomiasis. *Schistosoma japonicum* and *Schistosoma mansoni* cause hepatointestinal schistosomiasis in East Asia, Africa, South America, and the Caribbean, whereas *Schistosoma haematobium* causes urogenital schistosomiasis (UGS) throughout Africa and the Middle East and sporadically in Mediterranean Europe [1]. Recalibration of health burdens have revealed that schistosomiasis causes a loss of 4.5–70 million disability-adjusted life years [1], and estimates suggest that this neglected tropical disease rivals malaria in terms of socioeconomic impact. Of >110 million cases due to *S. haematobium* in sub-Saharan Africa, 70 million are associated with hematuria, 18 million with major bladder wall pathology, and 10 million with hydronephrosis leading to kidney damage [2]. The deposition of *S. haematobium* eggs eventually leads to squamous cell carcinoma of the bladder in some chronically infected individuals. Several studies in Africa have indicated a 2–10-fold relative risk in patients with schistosomiasis [3]. Accordingly, in one study a clinical history of UGS accounted for 16% of bladder cancer cases in Egypt. Consequently, *S. haematobium* is classified as a group 1 carcinogen by the International Agency for Research on Cancer [4]. Moreover, as many as 75% of women infected with *S. haematobium* have female genital schistosomiasis (FGS) [5]. FGS follows from the deposition of schistosome eggs in the uterus, cervix, vagina, and/or vulva, with ensuing inflammatory responses and increased susceptibility to human immunodeficiency virus infection

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and AIDS. Research directed toward understanding this pathogen has lagged, despite its global importance. Here, we highlight recent advances in the field that will facilitate the acquisition of deeper understanding of *S. haematobium* biology, its relationship with the host, and novel genomic tools that should assist in elucidating the role of targets for new interventions.

METHODS AND PRINCIPAL FINDINGS

Recent, major advances in the following areas will significantly facilitate discoveries in the pathophysiology of UGS: (1) comparative genomics, (2) functional genomics, and (3) tractable animal models to explore the interactions of *S. haematobium* with host systems (Figure 1). Access to the S. *haematobium* genome and transcriptome of male and female adult worms and eggs will allow comparative -omic studies [6]. Features of the *S. haematobium* genome that are conserved among members of Platyhelminthes and unique to *S. haematobium* are likely to provide new diagnostic and therapeutic targets for UGS. Moreover, RNA interference (RNAi) pathways in *S. haematobium* may be exploited to suppress key biomolecules for the viability, development, and infectivity of this pathogen [7].

GENOMIC SEQUENCING AND ANALYSIS—A MAJOR STEP FORWARD

Following the sequencing of the eukaryotic model organisms, including *Caenorhabditis elegans*, [8], improved, automated Sanger sequencing methods facilitated the characterization of the genomes of the first metazoan parasites, including *S. mansoni* and *S. japonicum*. These genomes provided the first, global previews of the genetics of schistosomes. In 2010, the completion of the draft genome of the giant panda, using solely Illumina/Solexa-based (massively parallel) sequencing [9], led to a revolution in the sequencing of an expanding catalog of metazoans, and sequencing of the *S. haematobium* genome ensued rapidly [6]. In this latter project, total genomic DNA from a single pair of worms was subjected to whole-genome amplification followed by sequencing; the genome was assembled and annotated using deep transcriptomic data from the egg and adult developmental stages [6]. The availability of the draft genomes of all 3 main species of schistosomes of humans has paved the way for extensive comparative [10] and functional genomics and for deep research of parasite-host interactions and pathogenesis [11].

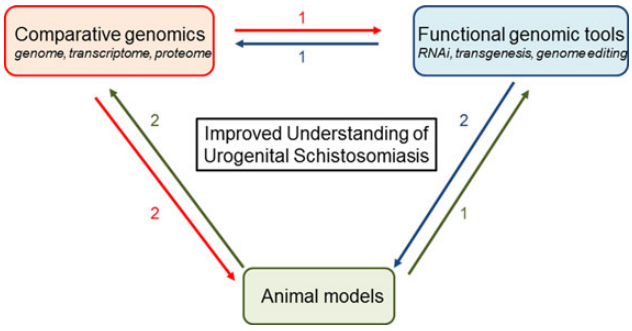

Figure 1. Improving understanding of urogenital schistosomiasis through comparative and functional genomics and animal models. Data from high-throughput sequencing (ie, genomic, transcriptomic, proteomic data) are investigated by functional genomic tools to assign roles to novel sequences. Transgenesis would also facilitate forward genetics by insertional mutagenesis that, in turn, can be analyzed by genomic approaches. Genetically modified parasites would be tested in tractable rodent models, and parasites obtained from these models would be studied using genomic approaches and functional tools. Hypotheses derived from comparative genomic approaches would be tested using functional genomic tools and animal models of urogenital schistosomiasis. Abbreviation: RNAi, RNA interference.

TOWARD ENHANCED ANNOTATION AND CURATED DATABASES

The functional annotation of schistosome genes has relied on homology-based bioinformatic analyses that assign gene annotation (s) from other species. This process infers that homologous proteins have conserved functions, although many schistosome proteins do not have orthologs in other taxa for which genomic, transcriptomic, and/or proteomic information are available. Thus, numerous schistosome proteins remain refractory to annotation; for example, of the 13 073 genes predicted for *S. haematobium*, only approximately 50% are homologous to curated sequences in the SwissProt:UniProt Knowledgebase [6] or are predicted to encode conserved amino acid sequence domains (InterPro) [6]. Furthermore, only approximately 40% of genes are linked to conserved biological pathways in the Kyoto Encyclopedia of Genes and Genomes [6] or processes inferred by gene ontology (GO) [6]. The platyhelminths, including the schistosomes, are Lophotrochozoa and distinct developmentally from members of the Ecdysozoa (eg, *Drosophila melanogaster* and *C. elegans*) and the Deuterostomia (eg, *Homo sapiens* and *Mus musculus*), whose genomes have been functionally annotated [8]. Therefore, the challenge of inferring the functional annotation of schistosome genes and proteins relates to their evolutionary distinctiveness from other organisms for which genomes have been well characterized.

Improvements in cross-taxon annotation can be achieved by integrating genomic and other -omics data [12]. In schistosomes, the first step was the curation of the annotated genomes of *S. haematobium*, *S. japonicum*, and *S. mansoni* within the public database SchistoDB [13]. The recent integration of data from these 3 species now enables informative data mining and definition of genes, GO terms, conserved domains, and biological pathways. Together with databases for other key flatworm

species, including GeneDB [14] and HelmDB [15], access is now available to an abundance of genomic, transcriptomic, and proteomic data to construct biological pathways and prioritize drug or vaccine candidates [16]. Furthermore, the identification of parasitic flatworm orthologs in the draft genomes and transcriptomes of free-living species of flatworms that have been established as models for stem cell regeneration could facilitate molecular investigations into the biology of *S. haematobium* [17]. To identify orthologs, SchistoDB and HelmDB contain protein-clustering algorithms to enhance the prediction of orthologs and paralogs. Therefore, whereas functional annotation of *S. haematobium* genes is limited, it is possible to confidently infer orthologs among the species of schistosomes. Methods used to define orthology/paralogy vary [18], but they all involve identification of orthologous genes/proteins shared by ≥2 species, usually based on pairwise sequence alignments [19]. Based on sequence homology (BLASTp; $E \leq 10^{-5}$), genome-wide comparisons of the inferred proteome of *S. haematobium* revealed that 1369, 244, and 10 880 proteins are homologous to those of *S. mansoni* alone, *S. japonicum* alone, and both species, respectively. Overall, pairwise comparisons of shared proteins among the schistosomes showed that most annotated biological pathways are shared [6]. Differences are evident among macromolecules associated with cell-cycle regulation, membrane transport and signaling, translation, and lipid/xenobiotic metabolism in *S. haematobium* and *S. mansoni*, compared with *S. japonicum*, and with transcription in *S. haematobium* and *S. japonicum*, compared with *S. mansoni*. In addition, a subset of 73 predicted enzymes and other proteins was unique to *S. haematobium* [6]. An OrthoMCL clustering approach [18] revealed that the schistosome proteomes shared 6968 orthologous clusters, of which 5389 were represented by single-copy genes. In addition, this approach identified orthologous gene clusters exclusive to *S. haematobium* and *S. mansoni* (1138 clusters; 989 single-copy genes) and to *S. haematobium* and *S. japonicum* (689 clusters; 654 single-copy genes). These findings indicate that further annotation of each gene set, using similar bioinformatic tools to characterize the kinome [20] and G-coupled protein receptors [21] of *S. mansoni,* will likely reveal differences in physiology among the 3 major species of schistosomes.

Curated genome annotations combined with advances in proteomics, such as through the characterization of tegumental and excretory/secretory proteins of schistosomes, can now be used to assemble a complementary data set of well-defined proteins for *S. haematobium*. The improved accessibility to genomic data of *S. haematobium* and other flatworms, the availability of enhanced bioinformatic tools, the standardization of annotation ontologies for genes, and the expanded functional annotation of sequences by protein clustering represent considerable advances that should expand the scope for systems biological studies of schistosomes.

FUNCTIONAL GENOMICS

Insights into the -omics of *S. haematobium* may be confirmed and expanded by functional testing of gene candidates, particularly those exhibiting novelty or evolutionary divergence among orthologs from the schistosome species. We anticipate that the transgenesis of *S. haematobium*, in addition to other genome manipulation approaches, will accelerate discoveries relevant to UGS by querying gene function over the developmental cycle, including in cercariae, schistosomula, adult worms, eggs, and sporocysts within the intermediate host snail [7]. Indeed, progress has been made in this area, and rapid advances can be predicted in development of functional genomic tools for forward and/or reverse genetic investigations of *S. haematobium* [6, 22, 23]. However, it has been more difficult to deploy transgenesis to *S. haematobium,* in comparison with *S. mansoni* and *S. japonicum*, largely because the developmental cycle of *S. haematobium* is challenging to maintain in laboratory mice.

To overcome these hurdles, Rinaldi et al [7] established protocols to culture several developmental stages of *S. haematobium* and demonstrated that square-wave electroporation and labeled small-molecule probes can be used to manipulate cultured developmental stages of *S. haematobium*. Cy3–small interfering RNAs (siRNAs) will enter eggs (Figure 2) and blood vessel stages of *S. haematobium*. In addition, firefly luciferase messenger RNA is translated in S. *haematobium* cells. Notably, these studies confirmed that the RNAi pathway is active in this schistosome; both a reporter transgene (luciferase) and an endogenous *S. haematobium* gene encoding the tegumental antigen tetraspanin 2 were posttranscriptionally silenced after specific RNA interfering molecules (siRNA and double-stranded RNA) were introduced into the egg, schistosomular, and adult stages of this blood fluke [7].

We are now attempting to establish stable transfection by using the integration competent vector pseudotyped murine leukemia virus retrovirus [24]. Other integration-competent vectors, including the transposon *piggyBac,* that can integrate into schistosome chromosomes will be investigated [25]; this approach also shows promise for germ-line transgenesis in other parasitic helminths [26]. *S. mansoni* transgenesis techniques (ie, use of *piggyBac* and retroviruses) are facilitating the development of forward genetics (ie, insertional mutagenesis analysis), reverse genetics, (ie, loss-of-function approaches), gain-of-function approaches, and overexpression of reporters (eg, genes encoding for antibiotic resistance). Thus, we anticipate that this technology will be transferable to *S. haematobium.* Table 1 presents a brief summary of the approaches and tools that have been modified and deployed for use in functional genomic studies of schistosomes. Although progress is farthest advanced in *S. mansoni*, there are no apparent physiological impediments that could thwart application to *S. haematobium* to derive stable transgenic lines (Figure 3) [27]. Figure 4

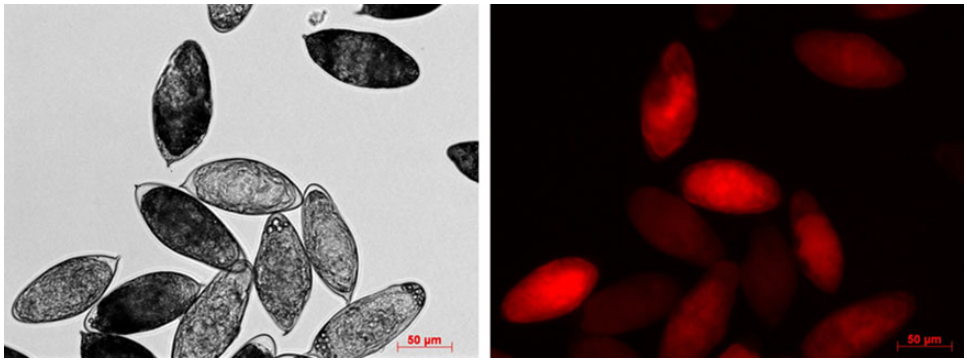

Figure 2. Labeled small interfering RNA (siRNA) enters *Schistosoma haematobium* eggs. Representative micrographs of eggs 3 hours after soaking in Cy3-siRNA. Left panel, eggs in medium containing 50 ng/µL Cy3-siRNA (bright field). Right panel, eggs in medium containing 50 ng/µL Cy3-siRNA (fluorescence field). Scale bar, 50 µm. Images from Rinaldi et al [7].

presents a schematic of how these approaches can be linked to propel advances in functional genomics for *S. haematobium*. Recently developed genome editing technologies that allow the introduction of site-specific mutations by using tailor-made nucleases, such as meganucleases, zinc finger nucleases, transcription activator–like effector nucleases, and clustered regularly interspaced short palindromic repeats–associated endonucleases (eg, Cas9), have been successfully applied to *C. elegans* to generate transgenic organisms and introduce site-specific, heritable mutations [28]. It is expected that this emerging technology will be transferable to parasitic helminths, thereby expanding the nascent tool kit for functional genomic investigations of schistosomes [29].

Table 1. Functional Genomics Manipulations and Tools Modified and Deployed With the Human Schistosomes

Functional Genomics Manipulation/Tool	*Schistosoma* Species	Reference[s][a]
RNAi using dsRNA, siRNA	*S. haematobium*, *S. japonicum*, *S. mansoni*	[50, 51]
Reporter gene activity	*S. haematobium*, *S. japonicum*, *S. mansoni*	[7, 52–56]
Chromosomal integration by (retro)transposon	*S. mansoni*	[25, 57]
Transgene delivered long/short hairpin RNAi	*S. mansoni*, *S. japonicum*	[58–60]
Rescue from antibiotic by resistance marker (neomycin phosphotransferase)	*S. mansoni*	[27]
Insulator activity to protect transgene (cHS4)	*S. mansoni*	[29]
Germ-line transgenesis to filial generations	*S. mansoni*	[27]
Transduction of in vitro laid eggs	*S. mansoni*	[27]

Abbreviations: dsRNA, double-stranded RNA; RNAi, RNA interference; siRNA, small interfering RNA

[a] Noninclusive list.

A relevant breakthrough was the identification of stem cells in schistosomes; Collins et al [30] reported neoblast-like stem cells in adult *S. mansoni*. This population of cells, distributed throughout the soma of male and female parasites, share not only morphology with the neoblasts of planarians, but also their ability to proliferate and differentiate into derivatives of multiple germ layers [30]. Based on a new cell isolation protocol for *S. mansoni* [31], it should be feasible to isolate these neoblast-like cells, target them in vitro with integration competent vectors such as transposons and retroviruses, and reintroduce transduced cells into larval stages as an alternative approach to derive stable, transgenic lines of schistosomes [27].

When established, these technological approaches should enable investigators to confidently address fundamental questions about the *S. haematobium*–mammalian host–parasite relationship, including the carcinogenic potential of *S. haematobium* eggs (particularly in tandem with the novel mouse model of egg-induced pathogenesis developed in Hsieh's laboratory [32]), host range, and tropism of this species for the pelvic rather than intestinal circulation. Some nonhuman primates are suitable hosts for *S. haematobium*, including patas [Erythrocebus patas] and vervet [Chlorocebus aethiops] monkeys and baboons [genus Papio] [33–41]. Application of genomics approaches (eg, genome editing) to primate models themselves would likely be informative [42].

Technological advances in the study of UGS should have translational value. The accurate detection of infection, the central goal of diagnostic tools for schistosomiasis, is based on analytes that vary in form and source (ie, protein vs nucleic acid, worm vs egg) across existing candidates vying for adoption. It is likely, however, that novel diagnostic tools that use markers from the *S. haematobium* genome will be based on nucleic acids or the protein products of characterized genes. These considerations also apply to diagnostic tools designed for detection of cercariae in water and sporocysts in snails [43].

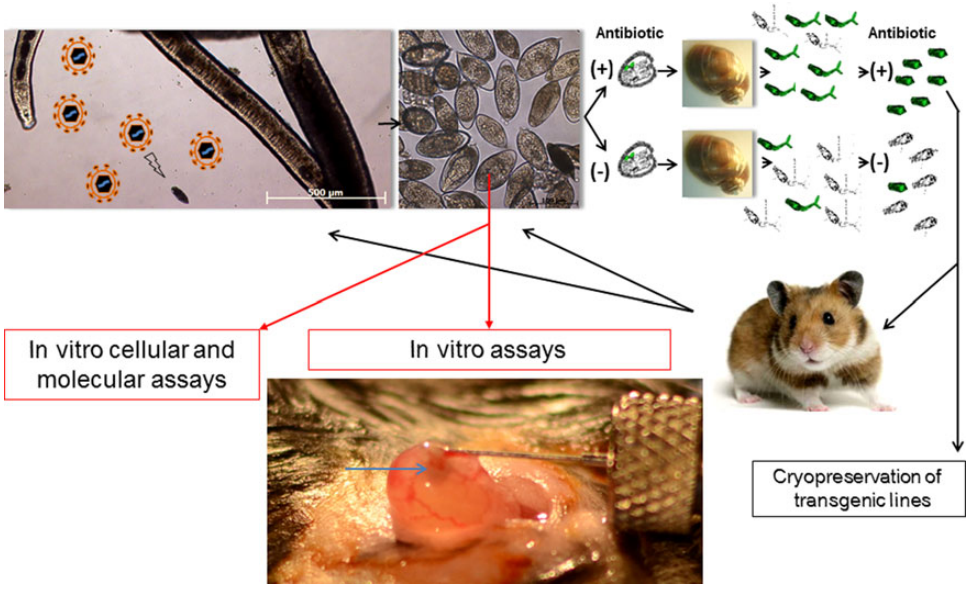

Figure 3. Cartoon depicting an approach to derive and maintain stable lines of transgenic schistosomes. A female schistosome releasing in vitro laid eggs exposed to murine leukemia virus retroviral virions (particles in upper left hand panel) and cultured with or without antibiotic. Miracidia hatched from eggs would be used to infect Bulinus truncatus snails. Cercariae cultured (through so-called in tandem antibiotic selection) with or without antibiotic could be screened for the presence of the transgene, for transgene copy number, and for expression. The transgenic line(s) could be propagated and maintained in hamsters. When the transgenic line emerges (eg, a line expressing an small hairpin RNA targeting a gene of interest), the eggs would be collected and analyzed in vitro by cellular and molecular assays and in vivo assays, including mouse models of urogenital schistosomiasis (adapted from Rinaldi et al [27]). Image under "In vivo assays" shows bladder wall injection with *Schistosoma haematobium* eggs (as described by Hsieh et al [32]). The egg bolus can be seen as a semiopaque bleb localized to the bladder wall (arrow in bottom panel).

Considering the translational potential of functional genomics of *S. haematobium,* it is informative to examine the developmental stages. For instance, candidate vaccines for UGS are likely to be based on findings derived from cercariae, schistosomules, and adult stages, since the goal of such vaccines is to break the cycle of infection [44]. In contrast, candidate drugs for UGS could target all of these life stages, including the egg, to prevent egg-induced pathology. Last, molecular characterization of the sporocyst stage may reveal new strategies for interrupting or disrupting the infection of snails by *S. haematobium*.

The potential for functional genomics of *S. haematobium* to improve diagnostic tools, drugs, and vaccines is representative of the intersection between population-based field studies and functional genomics. Fieldwork in UGS, in the form of genetic cataloging of *S. haematobium* strains, will also be relevant to functional genomics by facilitating improved annotation of parasite gene functions and interactions. Such studies will be critical to validating observations of host-parasite interactions obtained through Hsieh's egg injection mouse models.

HYPOTHESIS TESTING USING NEW ANIMAL MODELS OF UGS

Comparative and functional genomics of *S. haematobium* need to be framed in the context of host-parasite interactions. Although crucial to diagnosis and understanding of clinical disease, samples of blood and urine obtained from patients infected with *S. haematobium* leave open important disease pathogenesis questions. Our inability to access human or parasite tissue at sites of active infection hinders our understanding of early and ongoing events that contribute to the severe and chronic problems associated with UGS.

Our understanding of the fundamental immunology of the bladder mucosal surface is remarkably incomplete, particularly compared with that of other organ environments, such as the gut and lung. This obstacle highlights the gaps in knowledge of immune responses in the bladder. Whether the effects of infection with *S. mansoni* on the liver and intestine are analogous to processes occurring in the bladder during UGS is not known. Thus, it is critical to identify and characterize the acute changes initiated in mammalian bladder tissue in UGS and to establish how these processes are linked to morbidity over time. This is a key area of inquiry to improve the diagnosis and therapy of complications of UGS.

Historically, the primary in vivo models for UGS have been *S. haematobium* cercariae–infected hamsters and nonhuman primates [45]. Although these infections support the maturation of the parasite and oviposition, use of these animals as research models of human disease feature challenges. Hamsters (*Mesocricetus auratus*) exhibit low rates of pelvic organ infection,

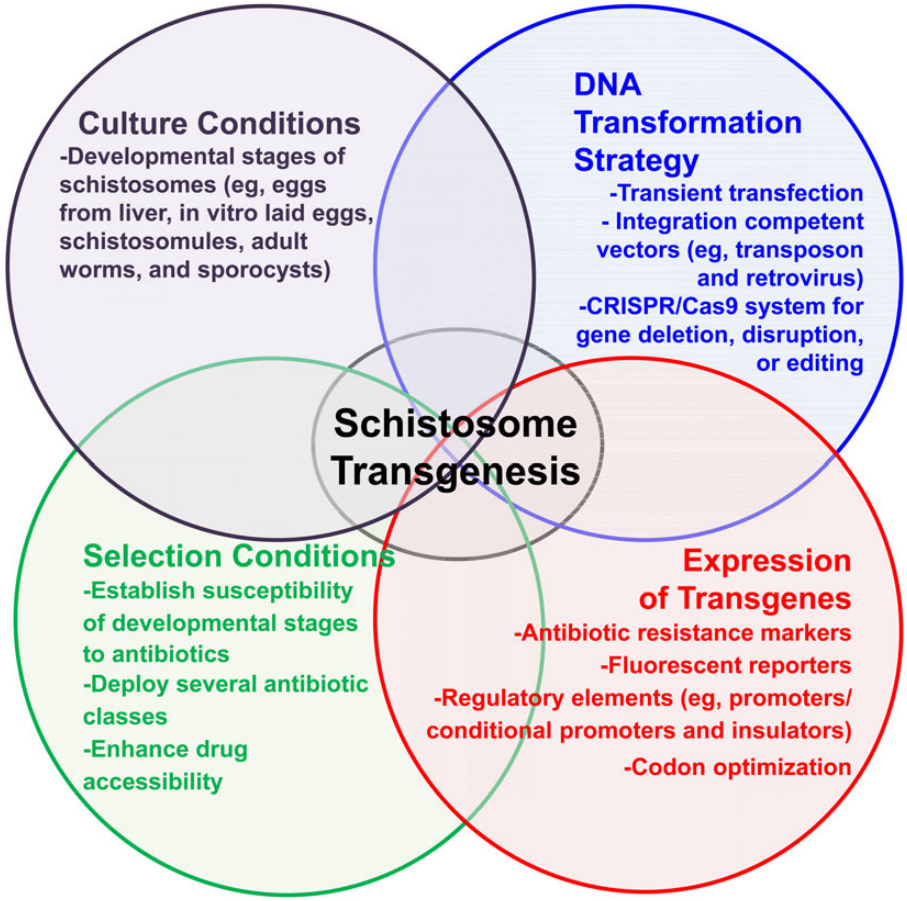

Figure 4. Methods to facilitate transgenesis for *Schistosoma haematobium*. General schema to establish transgenesis for schistosomes, in which (1) culture conditions of developmental stages, (2) genomic DNA transformation strategies (ie, retroviral transduction), and (3) stable expression of transgenes are linked to (4) specific selection conditions to specifically enrich the population of transgenic worms. Adapted from Chamberlin [61].

the key site of human pathology, and instead develop predominantly hepatoenteric schistosomiasis. Hence, while useful, the model is not amenable for studying disease and oviposition in pelvic organs. Moreover, there are few hamster-specific reagents and tools (eg, primary antibodies, cytokines, and transgenic strains) available for mechanistic studies. Nonhuman primates exhibit urogenital disease; however, use of these mammals requires substantial ethical justification, suffers from a lack of species-specific tools, and is expensive. Nevertheless, a number of nonhuman primates are receptive hosts for *S. haematobium* (ie, patas and vervet monkeys and baboons) [33–41]. Use of parasite- and host-specific genomics techniques should be possible with these animal models.

Although laboratory mice (*M. musculus*) are very amenable to *S. mansoni* and *S. japonicum* infections, attempts to transdermally infect mice with *S. haematobium* cercariae produce only light infections of the hepatoenteric systems and little or no oviposition in the pelvic organs [46]. An inability to model this key pathogenic phase of infection in mice is particularly unfortunate, given the wide range of species-specific tools that are available for mouse research. Therefore, until recently, approaches for modeling UGS in experimental animals have been largely unsuccessful.

We developed techniques that mimic the key pathological changes induced by *S. haematobium* oviposition in bladder tissue. We reasoned that direct administration of schistosome eggs into the mouse bladder wall could reproduce pathological elements of human bladder schistosomiasis. By analogy, our understanding of pulmonary fibrosis has been greatly aided by many studies of tail vein injection of *S. mansoni* eggs that lodge in the pulmonary capillary beds. Thus, administration of *S. haematobium* eggs directly into bladder tissue has the potential to be at least as relevant for understanding the pathogenesis of disease in the bladder.

Injection of *S. haematobium* eggs into the lamina propria of the mouse bladder—the tissue layer where egg granulomata are found in infected human bladders—recapitulated key aspects of the bladder disease of UGS [32]. Specifically, egg injection resulted in hematuria; increased urination frequency; the development of persistent, fibrotic bladder

granulomata; and systemic and regional type 2 immune activation [32].

A bolus injection of *S. haematobium* eggs is suitable for the controlled study of synchronized, egg-induced changes in host tissue. Microarray analysis of the whole bladder transcriptome by using this model revealed differential transcription of multiple genes relevant to inflammatory fibrosis and urothelial function [47]. This was the first characterization of the early transcriptional events occurring in the bladder after exposure to *S. haematobium* eggs. These data, derived from preparations of bladders, indicated the feasibility of detailing specific molecular, cellular, and biochemical events in subregions of the bladder and granulomata at serial times after egg injection. Such studies could elucidate the immunopathogenesis of UGS in the bladder mucosa and identify early inflammation-related events that contribute to onset of bladder fibrosis and schistosome-associated bladder cancer.

The bladder injection model in mice lays the foundation for tractable analysis of candidate pathogenic mechanisms used by *S. haematobium* eggs. Indeed, we have used this model to determine, for the first time, the immunologic basis of *S. haematobium*–infected host susceptibility to bacterial urinary tract coinfection [48]. Through the use of transgenic interleukin 4 (IL-4) receptor α–deficient mice and mouse-specific polymerase chain reaction, flow cytometry, and cytokine assays, we determined that *S. haematobium* egg–induced IL-4 suppresses invariant natural killer T-cell activation–associated clearance of bacterial urinary tract coinfection. This finding emphasizes the value of a mouse-based model of UGS.

Besides transgenic mice and other mouse-specific reagents, this model is also amenable to combination with transgenic schistosomes. For example, stably or transiently transgenic *S. haematobium* eggs or eggs subjected to exogenous RNAi could be injected, and their effects on host tissues could be compared with the effects of wild-type eggs. Cross-species comparisons could also be undertaken by injecting *S. mansoni* or *S. haematobium* eggs into mouse bladders, including in combination with transgenic/RNAi manipulations. Indeed, cross-species studies should facilitate the testing of hypotheses arising from comparative genomic analyses of the 3 human schistosomes, including the tropism for pelvic versus hepatointestinal organs. This model could also be used to address questions derived from population-based field studies in UGS, such as virulence and/or pathogenicity differences among natural *S. haematobium* strains, by injecting eggs from field strains into the bladder walls of mice.

Although these are new opportunities, the bladder-injection animal model has limitations. First, the approach permits study of egg-related phenomena only in host bladder tissue. Studies of the interaction between the host and cercariae, schistosomules, and adult worms will not be furthered by this technique. Regardless, much of the human pathological alterations arising from *S. haematobium* are directly related to egg deposition within bladder tissue. Thus, an egg-injection model remains valuable for inquiring about the pathogenesis of bladder-related disease in UGS. The injected eggs span a maturation spectrum, since they are isolated from chronically infected hamsters. This might introduce artifacts, since older or dead eggs are suddenly introduced into the bladder, rather than maturing and/or dying over time. Fortunately, it is feasible to collect eggs laid in vitro by the adult schistosome [49]. Newly laid eggs could be introduced into the bladder wall and may satisfactorily resemble a natural infection featuring deposition of more immature eggs. Another caveat is that stable, conditional, and/or long-term transgenesis, as well as gene silencing of *S. haematobium*, remains to be established. Transgenic blood flukes generated in vitro may modulate transgene expression in vivo. The transgenic techniques outlined above can, on the basis of experience in model species, be expected to improve over time and surmount these obstacles. Other limitations of this model include the fact that egg transmission and successful excretion may be difficult to examine, since egg shedding in urine after bolus injection into the bladder wall is not worm based, unlike natural human infection. The short life span of the mouse may not be conducive to studying human carcinogenesis. Species differences in genetics, immunity, anatomy, and endocrinology are also relevant.

CONCLUSIONS AND SIGNIFICANCE

Comparative genomics will generate hypotheses regarding mechanisms of pathogenesis that can be tested through genetic manipulation of *S. haematobium* in the context of wild-type and transgenic mouse models. The integrated use of these new technologies by teams of collaborating researchers can begin to elucidate how pathogen and host systems interact during this important tropical infection.

Notes

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