Review Article

Transfection of Platyhelminthes

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Flatworms are one of the most diverse groups within Lophotrochozoa with more than 20,000 known species, distributed worldwide in different ecosystems, from the free-living organisms in the seas and lakes to highly specialized parasites living in a variety of hosts, including humans. Several infections caused by flatworms are considered major neglected diseases affecting countries in the Americas, Asia, and Africa. For several decades, a particular interest on free-living flatworms was due to their ability to regenerate considerable portions of the body, implying the presence of germ cells that could be important for medicine. The relevance of reverse genetics for this group is clear; understanding the phenotypic characteristics of specific genes will shed light on developmental traits of free-living and parasite worms. The genetic manipulation of flatworms will allow learning more about the mechanisms for tissue regeneration, designing new and more effective anthelmintic drugs, and explaining the host-parasite molecular crosstalk so far partially inaccessible for experimentation. In this review, availability of transfection techniques is analyzed across flatworms, from the initial transient achievements to the stable manipulations now developed for free-living and parasite species.

1. Platyhelminth Transfection Studies

The phylum Platyhelminthes or flatworms represent one of the most diverse groups within Lophotrochozoa with about 20,000 species distributed worldwide including free-living and parasitic organism classified into 17 major groups [1, 2]. All these acelomate worms have bilateral symmetry; they are hermaphrodite with some exceptions and have a simple centralized nervous system and a mesodermal germ layer [3, 4]. Flatworms are characterized by a high degree of morphological diversity and reproduction modes (Table 1). The phenomenon of asexual reproduction that is uncommon in the animal kingdom occurs in all major groups of flatworms. This supports the presence of a population of totipotent stem cells called “neoblasts” in free-living worms and “germ or germinal cells” on flukes and tapeworms [4]. Several human infections caused by flatworms are considered major neglected tropical diseases (NTDs) by the World Health Organization: cysticercosis, schistosomiasis, fascioliasis, paragonimiasis, and echinococcosis [5].

Developing techniques to manipulate flatworms is a growing topic in contemporary research as judged by the number of reports published during the last decade [6]. Maintenance of parasite species under laboratory conditions has been challenging and genetic manipulation is still difficult [7]. However, since the 90s, attempts have been made to identify and characterize the regions controlling the expression of genes in several species of flatworms [8]. Due to the lack of a good expression system for heterologous genes in these organisms, several mammalian cell lines have been employed as transfection targets to identify functional promoters in flatworms [9, 10]. In this regard, the recently described genomes for several of these organisms, including the free-living planarian Schmidtea Mediterranean [11], and the parasites Schistosoma mansoni, S. japonicum [12, 13], Taenia solium [14, 15], Echinococcus granulosus, and E. multilocularis [15] represent a considerable advantage. Those genome projects allowed us to identify orthologous genes of each species and group and their functional promoters as well as to carry out in silico metagenomic studies. Transfection studies for each of the three groups of Platyhelminthes done so far are described in this short review.

1.1. Tricladida. Planarians have the capacity of regenerating complete worms from a small fragment of their bodies
Table 1: Main characteristics of the groups where genetic transfection has been achieved.

| Group       | Biologic interactions                  | Adult body  | Life cycle | Example of genus |
|-------------|----------------------------------------|-------------|------------|------------------|
| Tricladida  | Mostly free-living                     | Nonsegmented| Simple     | Dugesia, Schmidtea|
| Trematoda   | Endoparasites of invertebrates and vertebrates | Nonsegmented| Complex    | Fasciola, Schistosoma|
| Cestoda     | Endoparasites of vertebrates           | Segmented   | Complex    | Taenia, Echinococcus|

In 1981, Baguñà described a group of cells conferring these regenerative properties as “neoblasts” [16–18]. In order to understand the basis of tissue regeneration in these flatworms, several studies were conducted [18], which could represent a valuable contribution to human regenerative medicine [16] as well as to the establishment of stable germ cell lines useful in transfection studies [19]. However, it was not until the advent of the molecular biology and genetic tools that further investigation in this phenomenon was possible. Thus, in 1999 the Dglvs gene (Dugesia VASA-like) was reported as the first gene expressed in neoblasts [20] and, almost simultaneously, a successful application of RNA interference (RNAi) was reported [21]. Since then, several related neoblast genes have been described and strategies for transient transfections have been developed for Tricladida [16]. The most used method for introducing exogenous genes in the different stages of these organisms is microinjection, which is also frequently used for silencing genes such as Dijpum, nanos, β-catenin, ndk, DijFGFR1, and DijFGFR2 [16, 18, 22, 23]. This method, although highly efficient in adult flatworms, was very invasive for early developmental stages. More recently, a novel method for introducing exogenous materials into developing planarian embryos by nanosecond exposure of eggs to pulsed laser has been reported. This represents the first report of planarian embryos being genetically modified without compromising their normal development [17]. However, availability of suitable vectors for stable transfections is required to allow incorporation of exogenous genetic material into the genome of these organisms. For example, in the case of planarians three mobile elements (mariner, Hermes, and PiggyBac) have been introduced using the green fluorescent protein (EGFP: enhanced green fluorescent protein) as reporter gene, using microinjection followed by electroporation to transfect the parenchymal cells of adult flatworms [19]. Until now, the three transposons have shown good efficiency of integration into the genome in neoblast cells. PiggyBac and Hermes appear to be quite stable showing a good expression after eight months of transfection [19].

2. Digenean Trematodes

Trematode infections reach high prevalence in developing countries [5, 24]. The helminth infection with the largest global prevalence is schistosomiasis with 207 million cases worldwide, mainly caused by three species of blood flukes: S. haematobium, S. mansoni, and S. japonicum. In the case of trematodes, extensive studies on vaccines, drug development, and diagnostic methods are available [24]. Moreover, the complete genomes of S. mansoni and S. japonicum have been elucidated [12, 13]. Attempts of identifying genes and introducing heterologous genetic material have been carried out for more than a decade. New technologies have enabled success to identify, to silence, and to carry out transient transfections of several genes. Stable transfections have been achieved, allowing the approach to questions about the involvement of specific genes in disease pathogenesis or the identification of new target candidates for drug treatment [24]. Several reviews are available where the genomic history of schistosomes, including advances on transfection, is well organized [8, 10, 25–29]. Table 2 summarizes the progress in the transfection of S. mansoni and S. japonicum.

Other trematodes causing infections of high global prevalence (>40 million cases) [24], such as Clonorchis sinensis (liver fluke), Opisthorchis viverrini (liver fluke), Paragonimus spp (lung fluke), Fascioloopsis buski (intestinal fluke), and Fasciola hepatica (intestinal fluke), have not been successfully transfected; successful methodologies developed for S. mansoni could be adapted for these trematodes [27]. However, the promoter region of cathepsin I from F. hepatica has been characterized through transient transfection of mammalian Vero cells [30]. Another case is the Paragonimus westermani retrotransposon sequences belonging to three LTR (long terminal retrotransposons) retrotransposon families [31]. Two of these retrotransposon sequences appeared to maintain their mobile activities as suggested by the presence of mRNA transcripts [31]. The ability to integrate into the flatworm genome makes transposons and retrotransposons excellent candidates to develop stable transfections [32].

Three methods have been exploited for nucleic acid delivery into schistosomes [28]: biolistic (particle bombardment/gene gun), electroporation, and infectious retroviral vectors (Table 2). Electroporation has been considered as the most efficient method for transfection of sporocysts and schistosomules. However, biolistic has also been successfully used on miracidia and adults [33]. The choice of a delivery method depends on the organism and the life cycle stage under study. Moreover, experiences in schistosomes can also help to choose and adapt one transfection method on related organisms.

An application of transient transfection methodologies is the silencing of specific genes through RNAi, involving studies on worm viability, development, tegument physiology, egg development, signaling pathways, and drug discovery. Efficacy of RNAi can be influenced by the method of delivery: the more often used in schistosomes are soaking and electroporation [8] and the most frequently used RNAi in schistosomes is dsRNA (long double stranded), followed by siRNA (small interfering) [29]. The properties of each RNAi have been important to define their use; for example, it has been suggested that siRNA accumulates faster in certain tissues [50], whereas dsRNA is more stable to RNAsie digestion [51].
Table 2: Transfection of heterologous genes in Schistosomes.

| Species      | Agent and method                          | Promoter            | Reporter gene | Life stage transfected | Transfection type | References |
|--------------|-------------------------------------------|---------------------|---------------|------------------------|-------------------|------------|
| S. mansoni   | RNA and plasmid by particle bombardment   | Spliced Leader      | Luciferase    | Adult worm             | Transient         | [34]       |
|              | Plasmid by particle bombardment           | Hsp70               | GFP           | Adult worm and sporocysts | Transient         | [35]       |
|              | Plasmid by particle bombardment           | ER60                | GFP           | Female miracidia with sporocysts | Transient         | [36]       |
|              | Plasmid by particle bombardment           | SmCNA               | GFP           | Adult worm             | Transient         | [37]       |
|              | Plasmid by particle bombardment           | ER60                | GFP           | Adult worm             | Transient         | [38]       |
|              | RNA by electroporation                    | Hsp70               | Luciferase    | Miracidia              | Transient         | [39]       |
| S. japonicum | VSVG-pseudo MMLV plasmid by cation polybrene | SL and hsp70       | EGFP and Luciferase | Schistosomula         | Transient         | [42]       |
|              | Electroporation                           | SmACT1.1            | Luciferase    | Schistosomula          | Transient         | [43]       |
|              | PiggyBac by electroporation               | Actin and HSP70     | Luciferase    | Schistosomula          | Stable            | [44]       |
|              | VSVG-pseudo MMLV plasmid by lipofectamine | Sma-Zinc            | Luciferase    | Adult worm and schistosomula | Stable            | [45]       |
|              | RNA and VSVG-pseudo MMLV by electroporation | —                   | CY3 and Luciferase | Eggs                   | Stable            | [46]       |
|              | MLV pseudotyped plasmid by lipofectamine or polyethylenimine | MLV 5', Pol II, vasa-like, Actin, Pol III U6 | Luciferase and EGFP | Schistosomula, eggs, and adult worms | Stable | [47]       |
| S. japonicum | Plasmid by electroporation                | CMV                 | EGFP and Luciferase | Schistosomula and adult worm | Transient         | [48]       |
|              | VSVG-pseudo pan tropic retrovirus plasmid by cation polybrene | LTR                 | hTERT         | Schistosomula          | Stable            | [49]       |

SL: splice leader, hsp70: heat-shock protein 70, ER60: endoplasmic reticulum 60, SmCNA: Schistosoma mansoni calcineurin 1, CMV: cytomegalovirus, SmAct 1: Schistosoma mansoni actin 1, Sma-Zinc: Schistosoma mansoni Zinc finger protein, hTERT: human telomerase reverse transcriptase, VSVG: vesicular stomatitis virus glycoprotein, MMLV: Moloney murine leukemia retroviral, and LTR: retrovirus long terminal repeat.

In addition, dsRNA experiments are cheaper than the siRNAs counterpart [29, 51]; however, siRNAs can be more efficient inhibitors when multiple sequence oligonucleotides are used against the same target [8, 28, 29, 51, 52]. Developments of RNA silencing in schistosomes and other trematodes have accumulated during the last decade (Table 3).

Table 3 shows that although the most widely used RNAi is dsRNA gene silencing also can be efficiently achieved with siRNA [29]. The RNAi agent and the delivery method can be defined after the gene target and the stage of the parasites are selected. It is worth mentioning that initial attempts towards knocking down the expression of S. mansoni essential genes through in vivo administration of siRNA on infected hosts have produced encouraging results [53]. This strategy, that takes advantage of the low mRNA levels of the homologue gene in the host’s tissues (hypoxanthine-guanine phosphoribosyl transferase: HPRTase), is restricted in the case of other essential genes [54].

3. Cestodes

Among cestodes the most important infections in public health are cysticercosis and hydatosis or echinococcosis, with high global prevalence in endemic countries [5, 85]. In the case of these parasites, extensive studies on immunodiagnosis, drug and vaccine development, and so forth have been carried out [85–87]. However, transfection studies on cestodes have been scarce. An important development in the manipulation of these parasites is the isolation of germinal...
Table 3: RNA silencing in trematode parasites.

| Species         | Rnai       | Target gene                      | Life stage target                      | Silencing efficacy | References |
|-----------------|------------|----------------------------------|----------------------------------------|--------------------|------------|
|                 | dsRNA      | SGTPI and GAPDH                  | Miracidia and sporocyst                | 70–80% (t); 40% (p) | [55]       |
|                 | dsRNA      | SmCB1                            | Schistosomula                          | 10-fold (t)        | [56]       |
|                 | dsRNA      | SmCB1 and SmCB31                 | Cercariae and adult worms              | 80% (t)            | [57]       |
|                 | dsRNA and  | SmAP                             | Cercariae and adult worms              | >90% (t); >70% (p) | [58]       |
| siRNA           |            |                                  |                                        |                    |            |
|                 | siRNA      | SmRPNI/POH1                      | Schistosomula                          | 80% (t)            | [59]       |
|                 | dsRNA      | Cathepsin D                      | Schistosomula                          | 100% (t)           | [60]       |
| siRNA           |            | HGPRTase                         | Cercariae                              | ↓ 27% parasite load, 65% (t) | [53]       |
|                 | dsRNA      | SmLAP1 and SmLAP2                | Eggs                                   | ↓ 80% hatching     | [61]       |
|                 |            | 32 genes (antioxidants, transcription factors, cellular signaling, and metabolic enzymes) | Miracidia | Mobility, growth, and viability affected | [62] |
|                 | siRNA      | SmAP                             | Adult worms                            | 80% (t)            | [63]       |
|                 | dsRNA      | SmTK4                            | Adult worms                            | 17–63% (p)         | [64]       |
|                 | dsRNA      | SmAQP                            | Adult worms                            | 90–95% (t)         | [65]       |
|                 | dsRNA      | SmPAL                            | Adult worms                            | Inconsistent results | [66] |
|                 | siRNA      | SmAP                             | Adult worms                            | 80% (t)            | [63]       |
|                 | dsRNA      | Sm-NPP-1                         | Schistosomula and adult worms          | 55% (t)            | [72]       |
|                 | siRNA      | SmCD59                           | Schistosomula                          | 60% (t)            | [73]       |
|                 | dsRNA      | SmCaMK,K, SmJNK, SmERK1, SmERK2, and SmRas | Schistosomula | SmERK1 92% (t), SmERK2 56% (t), SmRas 42% (t) | [74] |
|                 | dsRNA      | SmACC-1 and SmACC-2              | Schistosomula                          | SmACC-1 60% (t), SmACC-2 90% (t) | [75] |
| siRNA           |            | SmAP, SmNPP-5, and SmATPDasel     | Schistosomula and Adult worms          | SmAP 90% (t), SmNPP-5 >90% (t), SmATPDasel 80% (t) | [54] |
|                 | siRNA      | Sm5HTR                           | Schistosomula and Adult worms          | Larvae: 100% (t) and ↓ 80% motility; adult male and female: 90% (t) and ↓ 60% motility, 80% (t) and ↓ 50% motility, respectively | [76] |
|                 | dsRNA      | SjGCP                            | Adult worms                            | 75% (t)            | [77]       |
| siRNA           |            | Mago Nashi                       | Schistosomula                          | 66–81% (t)         | [78]       |
|                 | dsRNA      | Prxs 1 and Prxs 2                | Schistosomula and adult worms          | ~20% (t)           | [79]       |
|                 | dsRNA      | (SHSP) SjP40                     | Adult worms                            | 80% (t)            | [80]       |
| siRNA           |            | SjAR (SiRNA1 and SiRNA2)         | Schistosomula                          | 48% (t) and 73% (t) | [81]       |

S. mansoni

S. japonicum
cells lines. For *T. crassiceps* it was possible to regenerate complete cysticerci from cellular clusters [88]; for *E. multilocularis* new metacestodes were regenerated from the germinal layer [89]; in the case of *E. granulosus*, the isolation and in vitro maintenance and propagation of germinal cells have been reported [90, 91]. The most significant development in the transfection of cestode parasites was achieved on *E. multilocularis* using axenic cultures of metacercoides. After some time in coculture with rat hepatocytes, the germinal cells formed a laminar layer and then clustered until the regeneration of the metacercoid vesicles [92]. The first attempts of a transient transfection were done by lipofection of germinal cells with a cyano-fluorescent gene as a reporter under the control of the acta promoter [93]. This indicates that the genetic manipulation of cestode parasites is currently under examination with the goal of developing reliable methodologies for stable transfection and in vitro maintenance of cell lines.

### 4. Conclusion

The new technologies for genetic manipulation and transgenesis have been used in trematode parasites, specifically in *S. mansoni* [29], which is a starting point for other flatworms. However, the progress in helminthology will be transformed from descriptive to more functional investigations. The need to develop methods for the production and in vitro cultivation of germ cell lines for genetic manipulation is emphasized [89].

### Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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### Table 3: Continued.

| Species       | RNAi         | Target gene                | Life stage target                      | Silencing efficacy | References   |
|---------------|--------------|----------------------------|----------------------------------------|--------------------|--------------|
| *S. haematobium* | siRNA and dsRNA | Luciferase and Sh-tsp-2 | Eggs, schistosomula, and adult worms | >75% (p) for both | [82]         |
| *F. hepatica*  | dsRNA        | FheCL and FheCB            | Metaceriae                             | FheCLI: 80% (t)    | [83]         |
|               | dsRNA        | FhlAP                      | Young larvae                           | >90% (p)           | [84]         |

SGTP: facilitated diffusion glucose transporter, GAPDH: glyceraldehyde-3-phosphate-dehydrogenase, SmCB: *Schistosoma mansoni* cathepsin B, SmAP: *Schistosoma mansoni* alkaline phosphatase, SmRPNII/POHII: *Schistosoma mansoni* proteasome subunit, HGPRTase: hypoxanthine-guanine phosphoribosyltransferase, SmLAP: *Schistosoma mansoni* leucine aminopeptidase, SmTIK4: *Schistosoma mansoni* SYK kinase, SmAQP: *Schistosoma mansoni* aquaporin gene, SmPAL: *Schistosoma mansoni* peptidylglycine alpha-amidating lyase, SmGTP: *Schistosoma mansoni* glucose transporter, SmCa: *Schistosoma mansoni* calmodulin sensing, Sm-NPP-I: *Schistosoma mansoni* neuropeptide precursor 1, SmCaMK: *Schistosoma mansoni* calmodulin-binding kinase, SmJNK: *Schistosoma mansoni* C-JUN-N-terminal kinase, SmERK: *Schistosoma mansoni* extracellular signal-regulated kinase, SmRAS: small GTPase superfamily, SmACC: *Schistosoma mansoni* acetylcholine-gated chloride channels, SmHTR: *Schistosoma mansoni* serotonin-activated G protein-coupled R, SjGCP: *Schistosoma japonicum* gynecophoral canal, Prxs: peroxiredoxin, Sip40: *Schistosoma japonicum* short heat shock protein, SjAR: *Schistosoma japonicum* aldose reductase, FheCL and FheCB: *Fasciola hepatica* cathepsin L and B, FhlAP: *Fasciola hepatica* leucine aminopeptidase, and sh-tsp-2: transcription of tetraspanin 2. (f): knockdown; (t): transcript; (p): protein.
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References

[1] R. J. Bobes, G. Fragoso, A. Fleury et al., “Evolution, molecular epidemiology and perspectives on the research of taeniid parasites with special emphasis on Taenia solium,” Infection, Genetics and Evolution, vol. 23, pp. 150–160, 2014.

[2] P. D. Olson, M. Zarowiecki, F. Kiss, and K. Brehm, “Cestode genomics—progress and prospects for advancing basic and applied aspects of flatworm biology,” Parasite Immunology, vol. 34, no. 2–3, pp. 130–150, 2012.

[3] J. M. Martín-Durán and B. Egger, “Developmental diversity in free-living flatworms,” EvoDevo, vol. 3, no. 1, article 7, 2012.

[4] M. Reuter and N. Kreshchenko, “Flatworm asexual multiplication implicates stem cells and regeneration,” Canadian Journal of Zoology, vol. 82, no. 2, pp. 334–356, 2004.

[5] P. J. Hotez, M. E. Bottazzi, C. Franco-Paredes, S. K. Ault, and M. R. Periago, “The neglected tropical diseases of Latin America and the Caribbean: a review of disease burden and distribution and a roadmap for control and elimination,” PLoS Neglected Tropical Diseases, vol. 2, no. 9, article e300, 2008.

[6] S. Beckmann and C. G. Grevelding, “Paving the way for transgenic schistosomes,” Parasitology, vol. 139, no. 5, pp. 651–668, 2012.

[7] J. E. Allen and R. M. Maiizels, “Diversity and dialogue in immunity to helminths,” Nature Reviews Immunology, vol. 11, no. 6, pp. 375–388, 2011.

[8] B. H. Kalinna and P. J. Brindley, “Manipulating the manipulators: advances in parasitic helmintihns transgenesis and RNAi,” Trends in Parasitology, vol. 23, no. 5, pp. 197–204, 2007.

[9] J. P. Boyle and T. P. Yoshino, “Gene manipulation in parasitic helminths,” International Journal for Parasitology, vol. 33, no. 11, pp. 1259–1268, 2003.

[10] C. G. Grevelding, “Transgenic flatworms,” Parasitic Flatworms: Molecular Biology, Biochemistry, Immunology and Physiology, pp. 149–173, 2006.

[11] S. M. C. Robb, E. Ross, and A. S. Alvarado, “SmedGD: the Schmidtea mediterranea genome database,” Nucleic Acids Research, vol. 36, no. 1, pp. D599–D606, 2008.

[12] M. Berriman, B. J. Haas, P. T. LoVerde et al., “The genome of the blood fluke Schistosoma mansoni,” Nature, vol. 460, no. 7253, pp. 352–358, 2009.

[13] G. Cheng and R. E. Davis, “Transgenic planarian lines obtained by electroporation using transposon-derived vectors and an eye-specific GFP marker,” Proceedings of the National Academy of Sciences of the United States of America, vol. 100, no. 2, pp. 14046–14051, 2003.

[14] N. Shibata, Y. Umesono, H. Orie, T. Sakurai, K. Watanabe, and K. Agata, “Expression of vasa(vas)-related genes in germline cells and totipotent somatic stem cells of planarians,” Developmental Biology, vol. 206, no. 1, pp. 73–87, 1999.

[15] A. Fire, S. Xu, M. K. Montgomery, S. A. Costas, S. E. Driver, and C. C. Mello, “Potent and specific genetic interference by double-stranded RNA in caenorhabditis elegans,” Nature, vol. 391, no. 6669, pp. 806–811, 1998.

[16] P. A. Newmark, Y. Wang, and T. Chong, “Germ cell specification and regeneration in planarians,” Cold Spring Harbor Symposium on Quantitative Biology, vol. 73, pp. 573–581, 2008.

[17] A. S. Alvarado and P. A. Newmark, “Double-stranded RNA specifically disrupts gene expression during planarian regeneration,” Proceedings of the National Academy of Sciences of the United States of America, vol. 96, no. 9, pp. 5049–5054, 1999.

[18] P. J. Hotez, P. J. Brindley, J. M. Bethony, C. H. King, E. J. Pearce, and J. Jacobson, “Helminth infections: the great neglected tropical diseases,” Journal of Clinical Investigation, vol. 118, no. 4, pp. 1311–1321, 2008.

[19] Y. N. Alrefai, T. I. Okatcha, D. E. Skinner, and P. J. Brindley, “Progress with schistosome transgenesis,” Memorias do Instituto Oswaldo Cruz, vol. 106, no. 7, pp. 785–793, 2011.

[20] P. J. Brindley and E. J. Pearce, “Genetic manipulation of schistosomes,” International Journal for Parasitology, vol. 37, no. 5, pp. 465–473, 2007.

[21] V. H. Mann, S. Suttiprapa, G. Rinaldi, and P. J. Brindley, “Establishing transgenic schistosomes,” Parasite Immunology, vol. 30, no. 4, pp. 215–221, 2008.

[22] S. Suttiprapa, G. Rinaldi, and P. J. Brindley, “Genetic manipulation of schistosomes—progress with integration competent vectors,” Parasitology, vol. 139, no. 5, pp. 641–650, 2012.

[23] Y.-A. Bae and Y. Kong, “Divergent long-terminal-repeat retrotransposon families in the genome of Paragonimus westermani,” The Korean Journal of Parasitology, vol. 41, no. 4, pp. 221–231, 2003.
M. E. Morales, V. H. Kunz, W. P. Jackstadt, H. Zahner, and C. G. Grevedling, “HSP70-controlled GFP expression in transiently transformed schistosomes,” *Molecular and Biochemical Parasitology*, vol. 120, no. 1, pp. 141–150, 2002.

V. Wippersteg, K. Kapp, W. Kunz, and C. G. Grevedling, “Characterisation of the cysteine protease ER60 in transgenic *Schistosoma mansoni* larvae,” *International Journal for Parasitology*, vol. 32, no. 10, pp. 1219–1224, 2002.

A. Rossi, V. Wippersteg, M.-Q. Klinkert, and C. G. Grevedling, “Cloning of 5′ and 3′ flanking regions of the *Schistosoma mansoni* calcineurin A gene and their characterisation in transiently transformed parasites,” *Molecular and Biochemical Parasitology*, vol. 130, no. 2, pp. 133–138, 2003.

V. Wippersteg, F. Ribeiro, S. Liedtke, J. R. Kusel, and C. G. Grevedling, “The uptake of Texas Red-BSA in the excretory system of schistosomes and its colocalisation with ER60 promoter-induced GFP in transiently transformed adult males,” *International Journal for Parasitology*, vol. 33, no. 11, pp. 1139–1143, 2003.

O. Heyers, A. K. Walduck, P. J. Brindley et al., “*Schistosoma mansoni* miracidia transformed by particle bombardment infect Biomphalaria glabrata snails and develop into transgenic sporocysts,” *Experimental Parasitology*, vol. 105, no. 2, pp. 174–178, 2003.

J. M. Correnti and E. J. Pearce, “Transgene expression in *Schistosoma mansoni*: introduction of RNA into *Schistosoma* cells by electroporation,” *Molecular and Biochemical Parasitology*, vol. 137, no. 1, pp. 75–79, 2004.

G. Cheng, L. Cohen, D. Ndegwa, and R. E. Davis, “The flatworm spliced leader 3'-terminal AUG as a translation initiation methionine,” *The Journal of Biological Chemistry*, vol. 281, no. 2, pp. 733–743, 2006.

K. J. Kines, V. H. Mann, M. E. Morales et al., “Transduction of *Schistosoma mansoni* by vesicular stomatitis virus glycoprotein-pseudotyped Moloney murine leukemia retrovirus,” *Experimental Parasitology*, vol. 112, no. 4, pp. 209–220, 2006.

J. M. Correnti, E. Jung, T. C. Freitas, and E. J. Pearce, “Transfection of *Schistosoma mansoni* by electroporation and the description of a new promoter sequence for transgene expression,” *International Journal for Parasitology*, vol. 37, no. 10, pp. 1107–1115, 2007.

M. E. Morales, V. H. Mann, K. J. Kines et al., “piggyBac transposon mediated transgenesis of the human blood fluke, *Schistosoma mansoni*,” *The FASEB Journal*, vol. 21, no. 13, pp. 3479–3489, 2007.

K. J. Kines, M. E. Morales, V. H. Mann, G. N. Gobert, and P. J. Brindley, “Integration of reporter transgenes into *Schistosoma mansoni* chromosmes mediated by pseudotyped murine leukemia virus,” *The FASEB Journal*, vol. 22, no. 8, pp. 2936–2948, 2008.

K. J. Kines, G. Rinaldi, T. I. Okatcha et al., “Electroporation facilitates introduction of reporter transgenes and virions into schistosome eggs,” *PLoS Neglected Tropical Diseases*, vol. 4, no. 2, article e593, 2010.

V. H. Mann, S. Suttiprapa, D. E. Skinner, P. J. Brindley, and G. Rinaldi, “Pseudotyped murine leukemia virus for schistosome transgenesis: approaches, methods and perspectives,” *Transgenic Research*, vol. 23, no. 3, pp. 539–556, 2014.

X.-S. Yuan, J.-L. Shen, X.-L. Wang et al., “*Schistosoma japonicum*: a method for transformation by electroporation,” *Experimental Parasitology*, vol. 111, no. 4, pp. 244–249, 2005.

S. Yang, P. J. Brindley, Q. Zeng et al., “Transduction of *Schistosoma japonicum* schistosomules with vesicular stomatitis virus glycoprotein pseudotyped murine leukemia retrovirus and expression of reporter human telomerase reverse transcriptase in the transgenic schistosomes,” *Molecular and Biochemical Parasitology*, vol. 174, no. 2, pp. 109–116, 2010.

E. H. Feinberg and C. P. Hunter, “Transport of dsRNA into cells by the transmembrane protein SID-1,” *Science*, vol. 301, no. 5639, pp. 1545–1547, 2003.

J. J. Dalzell, N. D. Warnock, P. McVeigh et al., “Considering RNAi experimental design in parasitic helminths,” *Parasitology*, vol. 139, no. 5, pp. 589–604, 2012.

G. N. Gobert, H. You, and D. P. McManus, “Gaining biological perspectives from schistosome genomes,” *Molecular and Biochemical Parasitology*, vol. 196, no. 1, pp. 21–28, 2014.

T. C. Pereira, V. D. B. Pascoal, R. B. Marchesi et al., “*Schistosoma mansoni*: evaluation of an RNAi-based treatment targeting HGPRTase gene,” *Experimental Parasitology*, vol. 118, no. 4, pp. 619–623, 2008.

A. A. Daidara, R. Bhardwaj, Y. B. Ali, and P. J. Skelly, “Schistosome tegumental ecto-apyrase (SmATPase1) degrades exogenous pro-inflammatory and pro-thrombotic nucleotides,” *PeerJ*, vol. 2, article e316, 2014.

J. P. Boyle, X.-J. Wu, C. B. Shoemaker, and T. P. Yoshino, “Using RNA interference to manipulate endogenous gene expression in *Schistosoma mansoni* sporocysts,” *Molecular and Biochemical Parasitology*, vol. 128, no. 2, pp. 205–215, 2003.

J. M. Correnti, P. J. Brindley, and E. J. Pearce, “Long-term suppression of cathepsin B levels by RNA interference retards schistosome growth,” *Molecular and Biochemical Parasitology*, vol. 143, no. 2, pp. 209–215, 2005.

G. Krautz-Peterson, M. Radwanska, D. Ndegwa, C. B. Shoemaker, and P. J. Skelly, “Optimizing gene suppression in schistosomes using RNA interference,” *Molecular and Biochemical Parasitology*, vol. 153, no. 2, pp. 194–202, 2007.

D. Ndegwa, G. Krautz-Peterson, and P. J. Skelly, “Protocols for gene silencing in schistosomes,” *Experimental Parasitology*, vol. 117, no. 3, pp. 284–291, 2007.

J. F. Nahban, F. El-Shehabi, N. Patocka, and P. Ribeiro, “The 26S proteasome in *Schistosoma mansoni*: bioinformatic analysis, developmental expression, and RNA interference (RNAi) studies,” *Experimental Parasitology*, vol. 117, no. 3, pp. 337–347, 2007.

M. E. Morales, G. Rinaldi, G. N. Gobert, K. J. Kines, J. F. Tort, and P. J. Brindley, “RNA interference of *Schistosoma mansoni* cathepsin D, the apical enzyme of the hemoglobin proteolysis cascade,” *Molecular and Biochemical Parasitology*, vol. 157, no. 2, pp. 160–168, 2008.

G. Rinaldi, M. E. Morales, Y. N. Alrefaei et al., “RNA interference targeting leucine aminopeptidase blocks hatching of *Schistosoma mansoni* eggs,” *Molecular and Biochemical Parasitology*, vol. 167, no. 2, pp. 118–126, 2009.

M. De Moraes Mourão, N. Dinguiiraud, G. R. Franco, and T. P. Yoshino, “Phenotypic screen of early-developing larvae of the blood fluke, *Schistosoma mansoni*, using RNA interference,” *PLoS Neglected Tropical Diseases*, vol. 3, no. 8, article e502, 2009.

G. Krautz-Peterson, R. Bhardwaj, Z. Faghiri, C. A. Tararam, and P. J. Skelly, “RNA interference in schistosomes: machinery and methodology,” *Parasitology*, vol. 137, no. 3, pp. 485–495, 2010.
S. Beckmann, C. Buro, C. Dissous, J. Hirzmann, and C. G. Grevelding, "The Syk kinase SmlTK4 of Schistosoma mansoni is involved in the regulation of spermatogenesis and oogenesis," *PLoS Pathogens*, vol. 6, no. 2, Article ID e1000769, 2010.

Z. Faghiri, S. M. R. Camargo, K. Huggel et al., "The tegument of the human parasitic worm Schistosoma mansoni as an excretory organ: The surface aquaporin SmAQP is a lactate transporter," *PLoS ONE*, vol. 5, no. 5, Article ID e010451, 2010.

L. E. Atkinson, P. McVeigh, M. J. Kimber et al., "A PAL for Schistosoma mansoni PHM," *Molecular and Biochemical Parasitology*, vol. 173, no. 2, pp. 97–106, 2010.

G. Krautz-Peterson, M. Simoes, Z. Faghiri et al., "Suppressing glucose transporter gene expression in schistosomes impairs parasite feeding and decreases survival in the mammalian host," *PLoS Pathogens*, vol. 6, no. 6, Article ID e000932, 2010.

S. Štefanic, J. Dvořák, M. Horn et al., "RNA interference in Schistosoma mansoni schistosomula: selectivity, sensitivity and operation for larger-scale screening," *PLoS Neglected Tropical Diseases*, vol. 4, no. 10, article e850, 2010.

A. S. Taft and T. P. Yoshino, "Cloning and functional characterization of two calmodulin genes during larval development in the parasitic flatworm Schistosoma mansoni," *Journal of Parasitology*, vol. 97, no. 1, pp. 72–81, 2011.

M. A. Ayuk, S. Suttiprapa, G. Rinaldi et al., "Molecular and Biochemical Parasitology* vol. 173, no. 2, pp. 97–106, 2010.

G. Krautz-Peterson, M. Simoes, Z. Faghiri et al., "Suppressing glucose transporter gene expression in schistosomes impairs parasite feeding and decreases survival in the mammalian host," *PLoS Pathogens*, vol. 6, no. 6, Article ID e000932, 2010.

S. Štefanic, J. Dvořák, M. Horn et al., "RNA interference in Schistosoma mansoni schistosomula: selectivity, sensitivity and operation for larger-scale screening," *PLoS Neglected Tropical Diseases*, vol. 4, no. 10, article e850, 2010.

A. S. Taft and T. P. Yoshino, "Cloning and functional characterization of two calmodulin genes during larval development in the parasitic flatworm Schistosoma mansoni," *Journal of Parasitology*, vol. 97, no. 1, pp. 72–81, 2011.

M. A. Ayuk, S. Suttiprapa, G. Rinaldi et al., "Molecular and Biochemical Parasitology* vol. 173, no. 2, pp. 97–106, 2010.

G. Krautz-Peterson, M. Simoes, Z. Faghiri et al., "Suppressing glucose transporter gene expression in schistosomes impairs parasite feeding and decreases survival in the mammalian host," *PLoS Pathogens*, vol. 6, no. 6, Article ID e000932, 2010.
attenuated suicide Listeria monocytogenes," *Nature Biotechnology*, vol. 16, no. 2, pp. 181–185, 1998.

[94] C. Mizukami, M. Spiliotis, B. Gottstein, K. Yagi, K. Katakura, and Y. Oku, “Gene silencing in Echinococcus multilocularis protoscoleces using RNA interference,” *Parasitology International*, vol. 59, no. 4, pp. 647–652, 2010.

[95] L. Pierson, A. Mousley, L. Devine, N. J. Marks, T. A. Day, and A. G. Maule, “RNA interference in a cestode reveals specific silencing of selected highly expressed gene transcripts,” *International Journal for Parasitology*, vol. 40, no. 5, pp. 605–615, 2010.