Review

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Knocking down schistosomes – promise for lentiviral transduction in parasites

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Underpinned by major advances in our understanding of the genomes of schistosomes, progress in the development of functional genomic tools is providing unique prospects to gain insights into the intricacies of the biology of these blood flukes, their host relationships, and the diseases that they cause. This article reviews some key applications of double-stranded RNA interference (RNAi) in *Schistosoma mansoni*, appraises delivery systems for transgenesis and stable gene silencing, considers ways of increasing efficiency and specificity of gene silencing, and discusses the prospects of using a lentivirus delivery system for future functional genomic–phenomic explorations of schistosomes and other parasites. The ability to achieve effective and stable gene perturbation in parasites has major biological implications and could facilitate the development of new interventions.

Schistosomes and the need for functional genomic–phenomic studies

Schistosomiasis is a major neglected tropical disease, affects ~300 million people globally, and is responsible for ~300 000 deaths each year [1–3]. This debilitating disease is caused by a chronic infection with one or more schistosomes (blood flukes) including *Schistosoma mansoni*, *Schistosoma japonicum*, and *Schistosoma haematobium*. No vaccines are available, and treatment relies mainly on the use of a single drug, praziquantel, to which drug resistance appears to be emerging [4]. In a complex life cycle (Figure 1), *S. mansoni* is transmitted from an infected aquatic snail (*Biomphalaria*) to humans via skin penetration. Following schistosomule migration, adult worms develop and dwell in intestinal and hepatic blood vessels. Eggs released from female worms become embedded in the liver parenchyma and intestinal wall where they trigger immune-mediated granuloma formation [5]; some eggs pass through this wall into the gut lumen and are then excreted into the environment to complete the life cycle. The granulomata that form in tissues are the principal cause of disease, ultimately leading to complications including fibrosis and periportal hypertension [6,7]. Although numerous studies (reviewed in [6]) have explored the pathogenesis

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Glossary

Dicer: RNase III enzyme that cleaves dsRNA to siRNA.
Drosha: RNase III enzyme that initiates processing of miRNAs by cleaving primary miRNA transcripts to stem-loop structures (pre-mature miRNA).
Genotoxicity: damage to the genomic information that can lead to mutagenesis and cancer.
Lentivirus: a member of the Retroviridae that can infect dividing and resting cells.
Long terminal repeat (LTR): identical sequences resulting from reverse transcription flanking proviral DNA that contain promoter/enhancer sequences and a termination signal.
MicroRNA (miRNA): small non-coding RNA involved in post-transcriptional regulation of gene expression.
miRNA-adapted short hairpin RNA (shRNAmir): synthetic short hairpin RNAs that contain the flanking regions of a natural miRNA such that they are expressed as primary miRNAs.
Mutagenesis: introduction of stable, heritable changes to the genetic information.
Omega-1: immunomodulatory protein that is secreted by *S. mansoni* eggs.
Oncogene: a gene with the potential to cause cancer.
Promoter: sequence in DNA that is recognized by RNA polymerase enzymes to initiate transcription of a gene.
Pseudotyping: altering envelope proteins of a virus to expand the range of host cells that can be infected (tissue tropism) by combination of virus vectors with foreign virus envelope proteins.
Retroviridae: a family of viruses with a single-stranded (ss) RNA genome that is replicated after reverse transcription to proviral DNA and subsequent integration into the genome of the host cell.
γ-Retrovirus: a member of the Retroviridae that can infect dividing cells.
Reverse transcription: process in that the enzyme reverse transcriptase uses a single-stranded RNA template to synthesize a ssDNA molecule referred to as complementary DNA (cDNA).
RNA interference: process of post-transcriptional regulation of gene expression that is induced by double-stranded (ds) RNA and leads to the degradation of mRNA or transcriptional regression.
RNA trigger: dsRNA molecule that induces RNA interference.
RNA-induced silencing complex (RISC): a multiprotein complex that incorporates mature miRNAs or one strand of siRNA and initiates cleavage of the mRNA at a sequence complementary to the miRNA/siRNA.
Seed region: nucleotide hexamer or heptamer of the mature miRNA sequence at positions 2–7 or 2–8, respectively.
Short hairpin RNA (shRNA): short RNA sequences with complementary sense and antisense regions that result in a stem-loop secondary structure resembling a hairpin.
Small interfering RNA (siRNA): short dsRNA of 20–25 bp in length that can trigger RNAi.
Transduction: utilizing a viral vector for the delivery of genetic information to a cell.
Transgenesis: process of transferring a new gene into a cell with a view of producing a cell expressing this gene.
Virion: a complete virus particle outside a host cell containing the virus genome and proteins protecting it (capsid) that can be surrounded by a lipid layer (envelope).
Virus: infectious nucleic acid that replicates inside living cells.

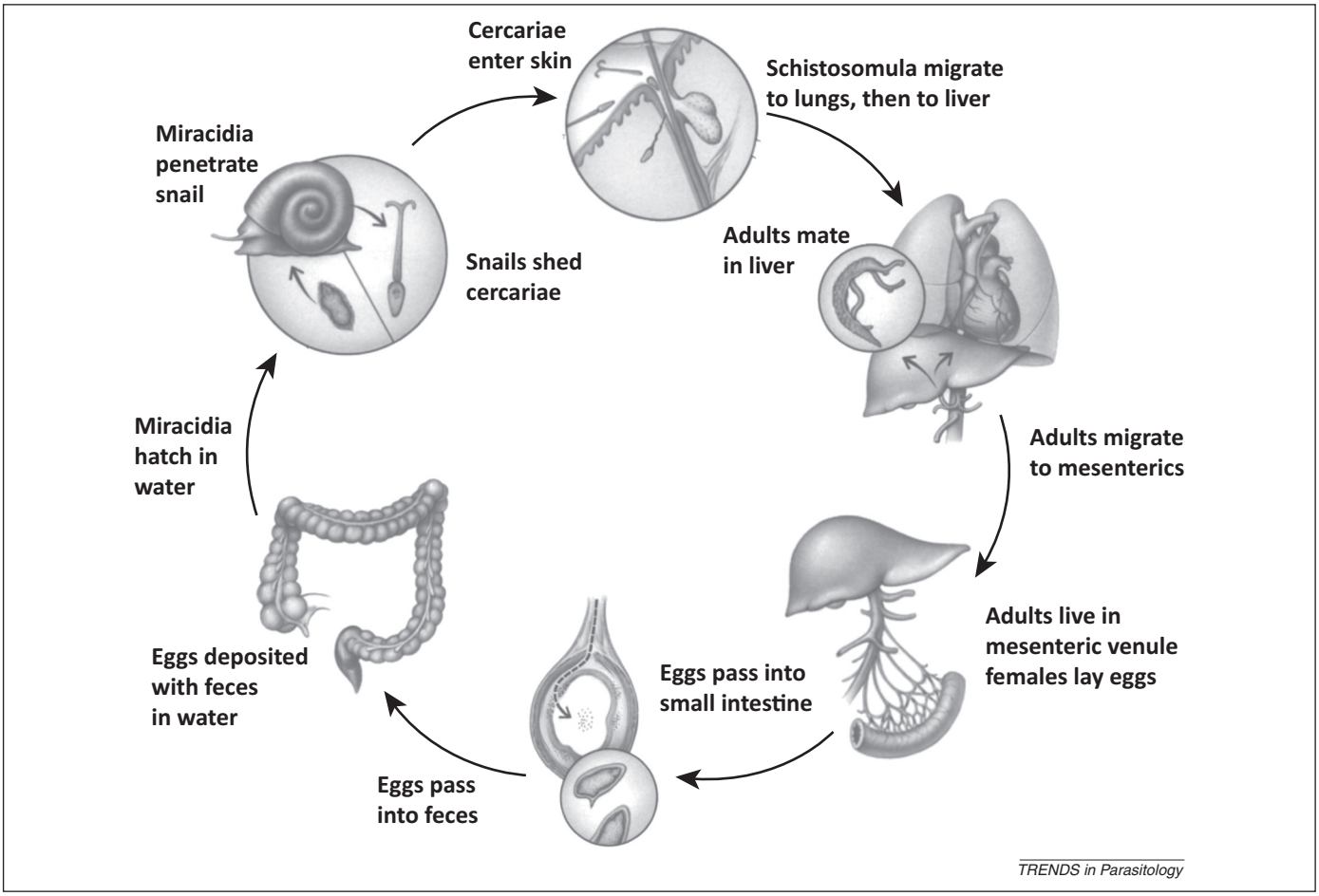

Figure 1. The life cycle of *Schistosoma mansoni*. Schistosome eggs are released in the feces from the human host. Upon contact with freshwater, miracidia hatch and penetrate the snail host (*Biomphalaria* spp.). Miracidia develop asexually into sporocysts in which further asexual propagation produces numerous cercariae. These motile stages actively penetrate the host skin, lose their tail, transform into schistosomula, and travel through the blood to the lungs and then the liver. After several days, schistosomula migrate to the portal venous system where they mature and unite. The adult worm pairs migrate to the mesenteric veins. Mature, gravid females release eggs, which pass into the lumen of excretory organs via induced inflammatory tissue-damaging processes. Modified from [94] with permission.

of the different forms of schistosomiasis, the molecular basis of the disease remains elusive. Knowledge gaps relate not only to the complexity of the biology of the parasites but also to technical obstacles.

Major advances in our understanding of schistosome genomes [8–10], and gradual progress in the development of functional genomic techniques (reviewed in [11–14]), are providing new and exciting opportunities to study and understand the intricacies of the schistosome–host relationship and the pathogenesis of disease. Various tools, such as RNA interference (RNAi; see Glossary) using double-stranded RNA (dsRNA) or small interfering RNA (siRNA), have been utilized to study the functions of single genes [12]. However, some methods employed to date can have limitations, such as off-target (non-specific) perturbation effects [15,16] and inadequate stability of gene knockdown, which can interfere with subsequent phenotypic assessment *in vitro* or *in vivo* in host animals [17]. These issues are compounded by the challenge of consistently producing sufficient amounts of the various parasite stages in the laboratory for functional genomic analyses [18]. The present article reviews some key applications of RNAi in *S. mansoni*, appraises the delivery systems for transgenesis and stable gene silencing, considers ways of increasing the specificity and efficiency of gene silencing, and discusses the prospects of using a lentivirus-based delivery system for future functional genomic–phenomic investigations of schistosomes and other parasites.

Applications of RNAi to *S. mansoni*

Following the first report of gene knockdown in schistosomes using synthetic long dsRNA [19], RNAi has been employed to study gene function in all life stages of *S. mansoni* (Figure 2). In conventional RNAi approaches, dsRNAs and siRNAs were usually delivered to the worms by soaking, electroporation, or particle bombardment. The processing of dsRNAs and longer hairpin RNAs (125–500 bp) results in multiple siRNA sequences (~21–25 nt) that can target different regions of the mRNA. Synthetic siRNAs can induce targeted gene knockdown at an efficiency comparable with dsRNAs [20]. However, the effectiveness of predicted siRNA requires experimental validation, which can be costly and time-consuming. The need to validate siRNAs before experimentation might be circumvented and the knockdown effect increased by using