Large-scale RNAi screening uncovers therapeutic targets in the parasite Schistosoma mansoni

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Schistosome parasites kill 250,000 people every year. Treatment of schistosomiasis relies on the drug praziquantel. Unfortunately, a scarcity of molecular tools has hindered the discovery of new drug targets. Here, we describe a large-scale RNA interference (RNAi) screen in adult Schistosoma mansoni that examined the function of 2216 genes. We identified 261 genes with phenotypes affecting neuromuscular function, tissue integrity, stem cell maintenance, and parasite survival. Leveraging these data, we prioritized compounds with activity against the parasites and uncovered a pair of protein kinases (TAO and STK25) that cooperate to maintain muscle-specific messenger RNA transcription. Large-scale RNAi screening uncovers therapeutic targets in the parasite Schistosoma mansoni.

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tudies of gene function in intramammalian schistosome parasites have been limited to relatively small numbers of genes (1). Therefore, we developed a large-scale RNA interference (RNAi) screening platform on adult schistosomes that prioritized a list of 2320 of the worm’s ~10,000 protein-encoding genes (fig. S1A and table S1). We generated double-stranded RNAs (dsRNAs) and treated adult male and female pairs with dsRNA over the course of a 30-day experiment (Fig. 1A). After filtering genes that either did not amplify by polymerase chain reaction or failed to generate sufficient concentrations of dsRNA, a total of 2216 genes were screened (table S1).

Schistosomes live in the veins surrounding the host intestines and attach to the vascular endothelium to avoid being swept away in the blood and trapped in host organs. Under in vitro culture conditions, healthy parasites attach to the substrate with a combination of their oral and ventral suckers (movie SI). We thus reasoned that substrate attachment would be a useful quantitative metric to define RNAi treatments that affect parasite vitality and to predict in vivo survival. Therefore, we monitored parasites for 30 days to identify substrate attachment and other visible defects.

Schistosomes possess adult somatic stem cells (neoblasts) that rejuvenate parasite tissues, including the intestine and tegument (skin) (2, 3). The parasites also contain large numbers of proliferative germline stem cells (GSCs) in their reproductive organs (2) that are essential for producing eggs, the central drivers of parasite-induced pathology in vivo (4). Therefore, we monitored the maintenance of neoblasts and GSCs by labeling parasites with the thymidine analog ethynyl deoxyuridine (EdU) before the conclusion of the experiment (Fig. 1A). Because of the variable rate at which the reproductive organs of female worms degenerate during in vitro culture (5), stem cell proliferation was only monitored in male worms. For genes with RNAi phenotypes uncovered during our screen, we confirmed gene identity by sequencing and mitigated potential off-target effects by designing an additional dsRNA targeting a nonoverlapping gene region or by examining sequence identity of hits with other Schistosoma mansoni genes (fig. S1). These studies identified 195 genes with fully penetrant attachment phenotypes, of which 121 possessed phenotypes in addition to attachment, including tissue and intestinal edema (36 genes), head (26) and/or tegument (78) degeneration, muscular hypercontraction (6), and death (36) (Fig. 1B and table S2). RNAi of an additional 66 genes resulted in stem cell maintenance defects but caused no other visible phenotypes (e.g., attachment), suggesting an essential role for stem cell maintenance (fig. S2 and table S3). We cannot rule out the possibility of false negatives among the genes with no phenotype and encourage greater scrutiny of such genes by alternate knockdown approaches or analysis of different phenotypic readouts.

Of the 66 genes required for stem cell survival, RNAi of over 90% (60 of 66) led to defects in the maintenance of both proliferative cells in the male testes and neoblasts (fig. S2). However, for a minority of genes, this maintenance defect appeared to be specific to either proliferative cells in the testes or neoblasts (fig. S2). Gene Ontology enrichment analysis identified genes important for protein translation, including gene products involved in ribosomal structure, tRNA aminoacylation, and ribosomal RNA (rRNA) processing as putative regulators of proliferative cell maintenance (fig. S3A). Although this could reflect an enhanced sensitivity of actively proliferating cells to alterations in protein translation, studies have highlighted “nonhousekeeping” roles for translational regulators in controlling stem cell function (6).

Of the 195 genes essential for parasite attachment, a large fraction share sequence similarity with other organisms, including other medically relevant schistosome species (table S4). Additionally, most genes with an attachment phenotype (172 of 195 genes; 88%) possess a close homolog from Caenorhabditis elegans or Drosophila melanogaster that likewise has a loss-of-function phenotype (table S5). Gene Ontology analyses of genes with attachment phenotypes further revealed that the dominant group of enriched genes were those encoding components necessary for protein turnover via the ubiquitin-proteasome system (UPS) (fig. S3B). Proteolysis is important for larval and adult viability in vitro (7, 8), and our data identified that key components from virtually every arm of the UPS were required for adult parasite viability during in vitro culture (fig. S4A). Indeed, RNAi of nearly all UPS components resulted in extensive tissue degeneration and in some cases adult parasite death (fig. S4, B and C).

To determine if any genes associated with attachment phenotypes encoded proteins targeted by existing pharmacological agents, we searched the literature and the ChEMBL database (table S6) (9). This analysis uncovered 205 compounds potentially targeting 49 S. mansoni proteins. We selected 14 of these compounds (table S7) and examined the activities of these compounds on worms cultured in vitro either by automated worm movement tracking or on the basis of parasite attachment. More than half of the compounds tested (8 of 14) on worms at 10 μM reduced parasite movement by >75%, and half of the compounds (7 of 14) caused fully penetrant substrate attachment defects by day 7 posttreatment (Fig. 2, A and B, and movie S2). Similarly, seven of these compounds affected the movement of postinfective larvae (schistosomula), suggesting activity against multiple life cycle stages (table S8).

Among the compounds that emerged from these studies was simvastatin, a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor with known effects on parasites both in vitro and in vivo (10). The proteasome inhibitor ixazomib affected both schistosome movement (Fig. 2A) and attachment (Fig. 2B), mirroring results in a recent report of the proteasome inhibitor bortezomib (7). However, the most potent effects on adult parasites were observed with CB-5083 and NMS-873, which inhibit the UPS component p97 by either competing with adenosine triphosphate
to be specific to inhibition of UPS function rather than a nonspecific effect due to reduced worm vitality (fig. S5D).

We then depleted UPS components using RNAi and surgically transplanted these worms into the mesenteric veins of recipient mice (fig. S6A), and we measured parasite egg deposition in host tissues and parasite survival. After hepatic portal perfusion, we recovered about 50% of transplanted control RNAi-treated worms (Fig. 2D and fig. S6B), and these parasites established patent infections, depositing large number of eggs into the livers of recipient mice (fig. 2E and fig. S6, C and D). By contrast, we failed to recover parasites after hepatic portal perfusion of mice receiving p97 (Fig. 2D) or proteasome subunit beta type-2 (fig. S6B). As a consequence, the livers in these mice were devoid of eggs, and we observed no signs of egg-induced granuloma (Fig. 2E and fig. S6, C and D). We also observed RNAi-treated parasites at various stages of infiltration by host immune cells (fig. S6, E and F). This suggests that these parasites are unable to remain in the portal vasculature and are cleared via the liver by the immune system. Taken together, these data highlight the essentiality and drugability of the schistosome UPS.

Another group of potentially druggable targets to emerge from our RNAi screen were protein kinases, 19 of which led to defects in either parasite attachment or stem cell maintenance. RNAi of two STE20 serine-threonine kinases, tao and stk25, which are homologs of the human TAO1/2/3 and STK25/YSK1 protein kinases, respectively, led to rapid detachment from the substrate (fig. S7A) and a concomitant posterior paralysis and hypercontraction of the body, such that the parasites took on a distinctive “banana”-shaped morphology (Fig. 3A, fig. S7B, and movie S4). Aside from RNAi of stk25 and tao, this banana-shaped phenotype of worms was distinctive, as it was only observed after RNAi of a CCM3/PDCD10 homolog (Smp_031950), a known heterodimerization partner with the mammalian STK25 kinase (14). We failed to observe death of either stk25- or tao-depleted parasites during in vitro culture. However, after surgical transplantation, we noted a reduction in the recovery of tao or stk25 RNAi-treated parasites from recipient mice, and these recipient mice displayed few signs of egg-induced granuloma formation (Fig. 3, B and C, and fig. S7, C and D). Thus, both tao and stk25 appear to be essential for schistosome survival in vivo.

Given the distinct and specific nature of the stk25- and tao-associated banana phenotype, we reasoned that these kinases may act in concert to mediate signaling processes in the worm. The Drosophila STK25 ortholog (GCK3) is a substrate of TAO, and these proteins function in a signaling cascade essential for tracheal development (15). Likewise, we observed that recombinant S. mansoni STK25

![Fig. 1. Summary of RNAi phenotypes. (A) RNAI treatment regimen. Parasites were monitored for visible abnormalities, and at day 29 EdU was added to medium to label proliferative cells. (B) Categories of RNAI phenotypes observed. kin-17 (Smp_023250), cog1 (Smp_132980), p97 (Smp_018240), c44 (Smp_136260), prp4b (Smp_068960), gtf2f1 (Smp_088460), stk25 (Smp_096640). Scale bar, 100 μm.](image)
Fig. 2. Compounds prioritized from RNAi studies have effects on schistosomes in vitro. (A) Effects on worm motility of compounds (red text) at 10 μM for 72 hours predicted to target schistosome proteins (blue text) essential for parasite attachment. n = 12 worm pairs, evaluated in three biological replicates. Data are means ± SD. Dashed line, threshold for 75% reduction in motility. DMSO, dimethyl sulfoxide (control). (B) Heat map showing time course for the fraction of male worms attached to the substrate after treatment with compounds for 72 hours as in (A). Three biological replicates were performed. (C) Effects of CB-5083 or NMS-873 (10 μM, 72 hours) treatment on male parasites. (D) Percent recovery of p97(RNAi) or control RNAi [cntl(RNAi)] male parasites surgically transplanted into mice. n = 8 transplants for each group. ****P < 0.0001. t test. Data are means ± 95% confidence intervals (CIs). (E) Hematoxylin and eosin staining of livers from mice receiving control or p97(RNAi) parasites. Yellow arrowheads indicate egg-induced granulomata. (Top right) Counts of eggs per square centimeter of liver section are shown (n = 3 livers). Scale bars, 100 μm [(C) and (E)].
Fig. 3. The protein kinases SmSTK25 and SmTAO are essential to maintain muscular function. (A) RNAi of stk25 or tao causes parasite hypercontraction. (B and C) Percent recovery of stk25(RNAi), tao(RNAi), or control(RNAi) male parasites surgically transplanted into mice. n = 11 transplants for each group. ****P < 0.0001, t test. Data are means ± 95% CIs. (D) Western blot to detect phospho-T173 (p-SmSTK25) or total SmSTK25 after an in vitro kinase reaction with or without ATP. Data represent results of two experiments. (E) Scatter plot showing the relationship between the differentially expressed genes [P(adj) < 0.001; Benjamini-Hochberg-corrected Wald test] after stk25 or tao RNAi treatment (r = 0.9, P < 0.0001; Pearson correlation). (F) Heat map showing expression of tissue-specific transcripts after RNAi of tao or stk25. (G) In situ hybridization for tropomyosin 1 (tpm1; Smp_340760) and a myosin light chain (ml2; Smp_132670) after RNAi at day 13. Scale bars, 500 μm (A) or 100 μm (G).

In situ hybridization to detect muscle-specific mRNAs. RNAi-treated parasites exhibited a reduction in the expression of mRNAs encoding the contractile proteins tropomyosin 1 and myosin light chain by in situ hybridization (Fig. 3G) but no qualitative defects in phallopoidin staining of body wall muscles (fig. S11). Thus, it appears that these kinases are required to maintain the transcription of many muscle-specific mRNAs in intact muscle cells. The heads of tao and stk25 RNAi parasites retained their capacity for movement (movie S4) and partially maintained the expression of muscle-specific mRNAs (Fig. 3G). Thus, there appears to be a relationship between the maintenance of muscle-specific mRNA expression and locomotion.

In their entirety, our data suggest that STK25 and TAO kinases cooperate in the schistosome to mediate signaling essential for sustaining the transcription of muscle-specific mRNAs. As a consequence, loss of either SmSTK25 or SmTAO activity results in muscular function defects compromising parasite survival in vivo. As whole-body knockouts of mouse STK25 are homozygous viable and display no obvious deleterious phenotypes (18), SmSTK25 represents an attractive target for therapeutic intervention. Similar cross-examination of genes associated with other phenotypic classes (e.g., tissue edema) may provide insights into the specificity of the various phenotypes observed in this work.

Technological advances have paved the way for large-scale analyses of gene function in protozoan parasites (19–21), but such analyses have been lacking for helminth parasites. Our systematic analysis of gene function in schistosomes allowed us to effectively prioritize targets and potential specific inhibitors with activities on worms. Future efforts should further explore the potency and selectivity of not only the compounds our studies have uncovered (Fig. 2, A and B, and table S6) but also larger libraries of compounds with known molecular targets (22). However, to mitigate potential issues with host toxicity, it is also worth exploring whether parasite-selective inhibitors of validated target proteins can be uncovered. Not only does this study enhance our understanding of schistosome biology, but also it provides a new lens to prioritize genes of interest in other medically and agriculturally important parasitic flatworms. Collectively, we anticipate such studies will expedite the discovery of new antihelminthics.
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Competing interests: The authors declare no competing interest.

Data and materials availability: Videos of RNAi attachment phenotypes can be accessed at www.collinslab.org/schistocyte or in (23). RNA sequencing analyses have been deposited in the NCBI Gene Expression Omnibus (GSE146720). 

SUPPLEMENTARY MATERIALS

science.sciencemag.org/content/369/6511/1649/suppl/DC1
Materials and Methods
Figs. S1 to S11
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References (24–60)
Movies S1 to S4
MDAR Reproducibility Checklist

View/request a protocol for this paper from Bio-protocol.

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