Schistosoma japonicum Proteins that Interact with the Gynecophoral Canal Protein Identified Using a Yeast Two-hybrid System

Yuqi Ren

Shanghai Veterinary Research Institute Chinese Academy of Agricultural Sciences

Mian Li

Henan Agricultural University

Yanli Shi

Northeast Agricultural University

Pingping Liu

Shanghai Veterinary Research Institute Chinese Academy of Agricultural Sciences

Qijin Wu

Yangzhou University

Yunxia Yang

Shanghai Veterinary Research Institute Chinese Academy of Agricultural Sciences

Longxian Zhang

Henan Agricultural University

Yamei Jin (✉ yameijin@shvri.ac.cn)

Shanghai Veterinary Research Institute Chinese Academy of Agricultural Sciences

Research

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Abstract

Background: The large amount of eggs produced by mature female worms not only induce major pathological damage to hosts of schistosomes but also lead to disease transmission. Mature female schistosome worms need constant pairing contact with a male partner as male signaling is indispensable to female growth, development, and reproduction. The gynecophoral canal protein (GCP), a cell-surface glycoprotein that is gender-specific to the male worm, plays a potential role in the interaction between males and females and in stimulating female development and maturation.

Methods: In this study, a yeast two-hybrid cDNA library of *Schistosoma japonicum* (Sj) parasites 18 days post-infection was constructed; the *SjGCP* gene was inserted into a pGBKT7-BD bait plasmid and used as a bait protein to screen for its molecular interactions using a yeast mating procedure.

Results: Twenty-four prey proteins that interacted with the SjGCP were selected after excluding false positives; the interactions between two of these, SjLGL and SjColV, and SjGCP were verified by co-immunoprecipitation.

Conclusions: The proteins that interacted with SjGCP were identified as being associated with growth, development, and reproductive functionality in *S. japonicum*.

Introduction

Schistosomiasis is an acute and chronic disease caused by blood flukes (trematode worms) of the genus *Schistosoma*, which results in severe pathological damage to both human and domestic animal hosts and causes substantial economic losses in many developing countries. Estimates show that at least 290.8 million people required preventive treatment for schistosomiasis in 2018 [1].

Unlike other trematode parasites, the blood flukes of the family Schistosomatidae are gonochorous; they have evolved separate sexes or dioecy, and the sex of an individual is determined in the zygote through a chromosomal mechanism [2]. Male schistosome worms are larger than the females, more muscular, and have a ventral groove called the gynecophoral canal in which females reside for maturation, mating, and egg production [3]. These dioecious worms exhibit unique reproductive biology: the female worms need to continuously pair with males to maintain sexual maturation [4, 5]. The stimuli from the male worms play important roles in the growth and reproductive development of female worms [6], and these stimuli are independent of sperm transfer and fertilization [4, 7–9]. The male worms are proposed to deliver specific molecules to female worms during pairing; the molecules associated this “magic male factor” have been unveiled in studies of the gynecophoral canal protein (GCP) [10].

GCP is a cell-surface glycoprotein that is specifically expressed in the gynecophoric canal of male schistosomes (the site of direct interaction between the mating pair) and widely distributed on the surface of mated females following transfer from the male [11–13]. Structurally, GCP lacks a transmembrane domain, but has short, conserved repeat regions with sequence similarity to Fasciclin I, a

developmentally regulated neural cell adhesion protein [13–16]. Fasciclin I-like proteins play important roles in cells, mediating adhesion, migration and differentiation [17–20]. Periostin, the mammalian homologue of Fasciclin I, also activates the Akt/PKB- and FAK-mediated signaling pathways [21, 22]. Interestingly, the transcription profile of GCP in schistosomes exhibits peak expression at a time that coincides with worm pairing [2]. Moreover, GCP is regulated via transforming growth factor- β (TGF- β)-dependent signaling in *Schistosoma mansoni* (Sm), providing evidence for a role of GCP in male-female interactions and the male-stimulated reproductive maturation of the female schistosome [23]. Studies of *S. japonicum* have revealed that GCP is necessary for pairing to occur between male and female worms [24]. Although the biochemical activity of GCP has not yet been clearly defined, there is accumulating evidence for its participation in male-female interaction in these worms [10].

Protein-protein interactions regulate many key biological processes, including cell-cell interactions, cell motility, signal transduction and vesicular trafficking and are fundamental to cell function [25, 26]. The yeast two hybrid (Y2H) assay is a technique that enables the rapid identification of several putative interacting proteins for a given protein of interest [27]. The great advantage of the Y2H assay, which is performed *in vivo*, is that the proteins being tested are more likely to be in their native conformations, leading to increased sensitivity and accuracy of detection [27, 28]. The Y2H system is both simple and highly efficient, and commonly used to screen for interacting proteins.

In the present study, we performed a Y2H screening procedure to identify proteins that interact with SjGCP, and the interactions were verified through yeast mating and co-immunoprecipitation.

Materials And Methods

Parasites and animals

The Chinese strain of *S. japonicum* was maintained in *Oncomelania hupensis* at the Shanghai Veterinary Research Institute, Chinese Academy of Agricultural Sciences (SVRI, CAAS). Freshly shed cercariae were obtained by exposing infected snails to light. The numbers and viability of the cercariae were determined prior to infection using a light microscope. Male New Zealand rabbits (6 weeks old and 2.0–2.5 kg, purchased from the Shanghai Experimental Animal Center, Chinese Academy of Sciences) were used to collect *S. japonicum* worms. To obtain the worms, the coverglass method of shaving the abdominal skin was used. The rabbits were each exposed to approximately 5,000 cercariae. The worms were collected from the infected rabbits at 18 days post-infection through hepatic-portal perfusion. This technique has been fully described previously [29]. The worms were washed three times in phosphate buffered saline (PBS, pH 7.4) at 4 °C to remove any residual host debris and stored in liquid nitrogen. The protocols of all animal experiments were approved by the Animal Care and Use Committee of the Shanghai Veterinary Research Institute, Chinese Academy of Agricultural Sciences (Shanghai, China; approval no: SYXK2011–0909).

Construction and identification of the pGBKT7-BD-SjGCP bait plasmid

The pGBKT7-BD-SjGCP bait plasmid was constructed and identified as previously described [30]. Briefly, total RNA was extracted from *S. japonicum* worms using TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA) and cDNA was reverse transcribed from the total RNA using a PrimeScript RT reagent kit (Takara, Japan). With reference to the *SjGCP* sequence in GenBank (accession no: AF519183), the open reading frame of the *SjGCP* gene was amplified by PCR from the cDNA, based on the specific primers (forward) 5'- ATCCATGGGCATGGAATCAATTCGGAATCACTCC-3' and (reverse) 5'- GTCCTGCAGCGTTTATATTCATCTTGAAATGGTG - 3'(the Nco I and Pst I restriction sites are underlined). The PCR product was ligated into the pGBKT7-BD plasmid digested with the same restriction enzymes. The recombinant pGBKT7-BD-SjGCP plasmid was confirmed by double restriction enzyme digestion and sequencing (Sangon Biotech, Shanghai, China) and retransformed into the yeast strain Y187 with the aid of the Yeastmaker Yeast Transformation System 2 kit according to the manufacturer's protocol (Clontech, USA). The recombinant pGBKT7-BD-SjGCP plasmid in the Y187 culture on SD/-Trp agar plates was extracted using an EasyYeast Plasmid Extraction kit (Clontech, USA) and resequenced.

The expression of BD-SjGCP fusion proteins in Y187 was analyzed by western blotting with Gal4 DNA-BD monoclonal antibodies. The auto-activation of the BD-SjGCP fusion protein was tested in Y187 and AH109 yeast cells on SD/-Trp/X- α -Gal, SD/-His/-Trp/ X- α -Gal and SD/-Ade/-Trp/X- α -Gal agar plates, and the toxicity of the BD-SjGCP fusion protein was analyzed by comparing the growth curve of the Y187 yeast cells with the pGBKT7-BD-SjGCP plasmid and an empty pGBKT7-BD vector.

Construction of a Y2H cDNA library for *S. japonicum* 18 days post-infection

Total RNA from *S. japonicum* 18 days post-infection was extracted using TRIzol reagent (Invitrogen, USA) and treated with DNaseI (Takara, Japan) to eliminate any contaminating genomic DNA. Then, a Y2H cDNA library was constructed using a homologous recombination method according to the instructions of the Matchmaker™ Library Construction and Screening Kit. Briefly, single stranded cDNA was synthesized from the 18 day total RNA using oligo dT primers, and double-stranded cDNA (ds cDNA) was obtained by LD-PCR amplification with 5' and 3' amplimers using an Advantage® 2 PCR Kit. The ds cDNA was further purified using a CHROMA-SPIN TE-400 column to limit very small inserts in the library. The purified ds cDNA and linearized pGADT7-Rec AD cloning vector with denatured Herring Testes Carrier DNA were transformed into competent AH109 yeast cells. The positive transformants cultured on SD/-Leu plates at 30 °C for 3–6 days were harvested to form the Y2H library. At the same time, transformants at dilutions of 10^1 , 10^2 , 10^3 and 10^4 were seeded on 100 mm SD/-Leu plates to calculate the transformation efficiency according to the following equation: transformation efficiency (cfu/ug DNA) = [clone number (cfu) $\times$ total suspension volume (μ L)] / [plating volume (μ L) $\times$ dilution factor $\times$ amount of

DNA used (ug)]. The harvested libraries from the 10¹, 10², 10³ and 10⁴ dilutions were then seeded on 100 mm SD/-Leu plates to calculate the library titer as follows: library titer (cfu/ml) = (colonies on SD/-Leu × dilution factor) / volume plated (ml).

To calculate the recombination rate of the library and analyze the average insert size, 96 colonies were randomly selected from the library and amplified by PCR with a Matchmaker LD-insert screening amplimer. The cycling conditions were as follows: 94 °C for 15 min, followed by 35 cycles at 94 °C for 1 min, 60 °C for 55 s, and 72 °C for 3 min. Specific primers (Table 1) for six known *S. japonicum* genes [*SjCyclophilin C* (GQ403664.1), *SjMagonashi* (GQ403668.1), *SjGCP* (AF519183), *SjCyclophilin B* (GQ403665.1), *SjZFP1* (GQ901167.1) and *Sj32* (AY429343.1)] were used to fish the target genes from the library to check the representativeness of the library.

Table 1
Primer sequences for the amplification of six *S. japonicum* genes

Gene names Accession No.	Primer sequence	Products (bp)
SjCyclophilin C GQ403664.1	Sense: 5'-ATGTCAACGTTTCCCAGGGT-3' Anti-sense:5'-TTAGCATTGCCCCGAATTAAC-3'	285
SjMagonashi GQ403668.1	Sense: 5'-ATGACGAGCTCATTTTATTT-3' Anti-sense:5'-TTATATTGGCTTTATTTTAA-3'	441
SjGCP AF519183.1	Sense: 5'-ATGGAATCAATTCGGAATCACTCC-3' Anti-sense:5'-TTAGGACTCAATAAGTGTAACCG-3'	1800
SjCyclophilin B GQ403665.1	Sense: 5'-ATGGCCGTGTTAAGAGTGTT-3' Anti-sense:5'-TCATTCAGCAGCGTCAGTGT-3'	642
SjZFP1 GQ901167.1	Sense: 5'- ATGGGTTTATGTAAGTG TGAA - 3' Anti-sense:5'- TCAAGCATCGTTTAACCCATC - 3'	1030
Sj32 AY429343.1	Sense: 5'- GAAAACGTTGATGATAG-3' Anti-sense:5'- GTATCTTTAATGAGATCCCTTC-3'	971

SjGCP interactive protein screening

The yeast mating method was used to screen for proteins that interact with SjGCP. Briefly, the AH109 yeast strain containing the cDNA library from 18 day *S. japonicum* was co-cultured with a Y187 yeast strain with the pGBKT7-BD-SjGCP bait plasmid. The mated culture was plated on SD/-Ade/-His/-Leu/-Trp (QDO) agar plates and incubated for 3–5 days at 30 °C. Simultaneously, 100 µl of the transformants at

dilutions of 10^1 , 10^2 , 10^3 and 10^4 were seeded on SD/-Leu, SD/-Trp and SD/-Leu/-Trp 100 mm agar plates to calculate the mating efficiency and screened clones as follows: mating efficiency = (cfu/mL of diploids $\times$ 100)/(cfu/mL of AH109 partner); screened clones = cfu/mL of diploids $\times$ resuspension volume (mL). Then, the director clone on QDO agar plates was passaged on QDO-supplemented X- α -Gal (QDO/X- α -Gal) agar plates three times for screening.

The monoclonal clones selected by repeated passages on the QDO-supplemented X- α -Gal agar plates were picked out, and the bait and prey protein expression plasmids were extracted using the yeast plasmid extraction kit. Yeast plasmids were identified by PCR using pGADT7 universal vector primers, and those that met the requirements were further transformed into *E. coli* DH5 α competent cells and then selected on LB agar plates (containing 100 μ g/ml ampicillin) to enrich the prey plasmids for sequencing. The obtained sequences were subjected to a BLAST search against the National Center for Biotechnology Information (NCBI) databases to annotate the functions of the corresponding genes.

Retesting the interactions in yeast by yeast mating

Reverse-hybridization was used to confirm the interaction between SjGCP and the potential prey proteins. Competent cells of yeast Y187 and AH109 were prepared according to the Clontech protocol, and the bait plasmid pGBKT7-BD-SjGCP, unrelated protein plasmid pGBKT7-BD-Lamin and empty vector plasmids pGBKT7-BD and pGBKT7-BD-53 were transferred into yeast Y187 cells, plated on SD-Trp culture plates, and cultured at 30 °C for 3–6 days. Each potential prey protein plasmid (pGADT7-AD-prey protein) as well as the empty vectors pGADT7-AD, pGADT7-AD-Lamin C and pGADT7-AD-RecT were transformed into yeast AH109 cells, plated on SD-Leu culture plates, and cultured at 30 °C for 3–6 days. Monoclonal AH109 and Y187 yeast cells grown on culture plates were mated in a 0.5 ml culture medium of 2 $\times$ YPDA, shaken at 30 °C for 20–24 h and the mating mixtures were cultured on SD/-Leu/-Trp plates for 3–6 days (Table 2). The diploid clones grown on the SD/-Leu/-Trp plates were then streaked on SQDO/X- α -Gal and cultured at 30 °C for 5 ~ 6 days.

Table 2
Repeat two-hybrid assay

	DNA binding hybrid(ah109)	Activation hybrid(Y187)
1	pGADT7-AD-prey protein	pGBKT7-BD-SjGCP
2	pGADT7-AD-prey protein	pGBKT7-BD
3	pGADT7-AD-prey protein	pGBKT7-BD-Lamin C
4	pGBKT7-BD-SjGCP	pGADT7-AD
5	pGBKT7-BD-SjGCP	pGADT7-AD-Lamin C
6	pGBKT7-53	pGADT7-RecT

Retesting the interaction in mammalian cells by co-immunoprecipitation

To further validate the interaction of SjGCP with selected target proteins, the prey protein's gene sequence was subcloned into pCMV-Myc using the enzymes Sfi I and Xho I from the pGADT7-AD-Prey to construct pCMV-Myc-Prey. The open reading frame of SjGCP was cloned into the p3xFLAG-CMV plasmid to obtain p3xFLAG-CMV-SjGCP. The pGADT7-AD-Prey and p3xFLAG-CMV-SjGCP plasmids were then co-transferred into HEK293 cells. Meanwhile, pCMV-Myc-Prey and p3xFLAG-CMV, and p3xFLAG-CMV-GCP and pCMV-Myc, were co-transferred into HEK293 as negative controls. The expression of the prey protein and SjGCP (and the interaction between them) in the HEK293 cells was detected by co-immunoprecipitation (Table 2). Briefly, after 48 h of culture, the HEK293 cells were harvested using non-denaturing cell lysis buffer. The supernatant was transferred to new tubes and the concentration of the total protein was determined using a BCA assay. The total protein (500 µg) was mixed with 10 µg of primary Red anti-Flag M2 beads and shaken on a rotating shaker at 4 °C overnight. Finally, the beads and immunocomplexes were washed with PBS, eluted by boiling in sample buffer and detected by western blotting using anti-Flag monoclonal mouse antibody (1:1000) and anti-Myc monoclonal mouse antibody (1:1000). Twenty micrograms of total protein were used as the input.

Results

Construction and identification of the pGBKT7-BD-SjGCP bait plasmid

The complete coding sequence of SjGCP (1,933 bp) was obtained by PCR amplification. Subsequently, the amplified fragments were subcloned into the vector pGBKT7 using Nco I and Pst I. The recombinant plasmid pGBKT7-BD-SjGCP was retransformed into Y187 and cultured on defective culture plates with SD/-Trp. Western blot analysis indicated that the BD-SjGCP fusion proteins were successfully expressed in Y187 yeast cells, did not exhibit auto-activity in Y187 and AH109 yeast cells and were not harmful to the yeast cells [31].

cDNA library construction *S. japonicum* at 18 days post-infection

The high integrity total RNA (A 260/A 280: 1.86A) extracted from 18-day post-infection *S. japonicum* were used to construct the Y2H cDNA library. The transformation efficiency of this library was 1.07×10^5 cfu/ug PGADT7-Rec and the library capacity was 2.5×10^6 pfu/mL. Ninety-six positive clones were randomly selected and identified by PCR using the Matchmaker LD-insert screening amplimer. The results indicated that the majority of the inserts ranged from 0.25 to 2.0 kb in size (Fig. 1). After purification using a Chroma Spin TE-400 column, the fragments smaller than 200 bp were eliminated, which

effectively avoided the presence of very small inserts and non-recombinant clones in the library. All six gene-specific primers were able to amplify the target genes from the library (Fig. 2). One *S. japonicum* eggshell protein gene (the messenger RNA of which should be in mature female worms) was not amplified from this library (data not shown). These results showed that the library was well represented and met the requirements for the subsequent Y2H library screening.

SjGCP interactive protein screening

The cultures mated with the library strain (AH109) and reporter strain (Y187) were plated on 100 mm QDO plates and SD/-Leu, SD/-Trp and SD/-Leu/-Trp plates at dilutions of 10^1 , 10^2 , 10^3 and 10^4 . The colonies were calculated, and the mating efficiency was found to be 20.92%; the number of screened clones was 4.1×10^7 cfu. A total of 522 colonies from the QDO plates were repeatedly passaged on QDO/X- α -Gal agar plates, and 383 mated clones were obtained. The yeast mating efficiency was determined to be 20.92% and the number of screened clones was 4.1×10^7 cfu.

The pGADT7-AD-prey plasmids from the 383 mated clones were extracted and identified by PCR using pGADT7 universal vector primers. They were re-transfected into DH5 and the 131 prey plasmids enriched in *E. coli* were sequenced and revealed exogenous insertion sequences. A total of 45 unique gene sequences were obtained after bioinformatic analysis.

Retesting the protein interactions in yeast by yeast mating

In order to exclude false positives, the pGADT7-AD-prey plasmid corresponding with these 45 unique gene sequences were re-transfected into AH109 yeast cells to reverse-hybridize with Y187 yeast cells that included pGBKT7-BD-SjGCP to verify their interactions. The interactions between SjLGL and SjGCP are shown in Fig. 3. Finally, 24 proteins that interacted with SjGCP in the yeast system were identified (Table 3).

Table 3
SjGCP interactive proteins selected using the yeast two-hybrid system

Protein and Gene Accession No.	Protein Name
SJCHGC00058 AAW27892.1	cathepsin B
SJCHGC00472 AAW26001.1	enolase
SJCHGC00694 AAW25358.1	Actin
SJCHGC06072 AAW26617.1	Twinfilin, actin-binding protein
SJCHGC09481 AAW27166.1	Calcium regulated heat stable protein
SJCHGC06673 AAX26676.1	calcium binding protein Sj66
SJCHGC06074 AAW27565.1	UDP-glucose 4-epimerase
SJCHGC05668 AAW26269.1	membrane-associated protein 29
SJCHGC06122 AGW24287.1	lethal giant larvae homolog protein
SJCHGC00443 AAA64426.1	fatty acid binding protein
SJCHGC09129 AAW24883.1	heat shock protein HSP60
SJCHGC05222 AAP04515.2	ubiquitin-conjugating enzyme E
SJCHGC09290 AAW27730.1	collagen alpha 1 (V)
SJCHGC05265 AAW24953.1	collagen alpha-1(III)
SJCHGC09378 AAX25986.2	COL3A1 protein
SJCHGC00545 AAW25240.1	Collagen alpha-2(V)
SJCHGC04226 CAX69383.1	Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 3
SJCHGC06185 TNN13299.1	Exosome complex component RRP41
SJCHGC05022 AAW25684.1	Proteasome subunit alpha type-6
SJCHGC08003 TNN09092.1	Nucleoporin Nup43 isoform 2
SJCHGC07184 AAX25303.2	Uncharacterized protein
SJCHGC05542 AAX24328.2	Uncharacterized protein
SJCHGC09800 AAX31087.1	Uncharacterized protein
SJCHGC06549 AAW25882.1	Uncharacterized protein

Retesting the protein interactions in mammalian cells by co-immunoprecipitation

To further determine whether the interaction between the prey and SjGCP occurred *in vitro*, co-immunoprecipitation was performed in HEK293T cells co-expressing SjGCP-FLAG and prey-Myc. The recombinant plasmid pCMV-Myc-prey was constructed using Sfi I and Xho I, and SjGCP was cloned into the p3xFLAG-CMV plasmid (Fig. 4A). Two prey proteins, SJCHGC06122 (SjLGL) and SJCHGC09290 (SjCol V) were selected to test their interaction with SjGCP. The results showed that SjGCP-FLAG could be co-immunoprecipitated with anti-FLAG antibodies or anti-Myc when co-expressed with SjLGL-Myc, demonstrating an interaction between SjGCP-FLAG and SjLGL-Myc in the cells (Fig. 4B).

Discussion

Schistosomiasis is one of the most important parasitic diseases in the world in terms of public health impact, second only to malaria. There are five schistosome species most often associated with human infections; the three clinically most important and highly studied species are *S. mansoni*, *S. haematobium* and *S. japonicum* [32, 33]. The major pathological damage induced by schistosomiasis is caused by the large amount eggs deposited in the host's tissues. These eggs can induce severe granulomatous and cause transmission of schistosomiasis following their release into the environment [34]. Therefore, blocking the maturation and oviposition of female worms is particularly important to the control of schistosomiasis.

As dioecious parasites, female schistosomes must maintain constant pairing contact with a male partner to induce differentiation of their reproductive organs. The co-clasping of male and female schistosomes is key to the sexual maturation of females, and male stimuli are indispensable to the growth and development of females [35]. Female worms that have been recovered from unisexual infections display stunted growth and undeveloped reproductive systems when compared with their counterparts from bisexual infections [8, 36]. Separation of established mating pairs results in the rapid degeneration of the vitelline gland and ovary, a decline in DNA synthesis associated with vitelline cell production and a reduction in the size of the female parasite [7], suggesting that schistosomes have developed a system by which male signals either directly or indirectly control female development and maturation [35, 37–41]. All of these phenomena indicate that male and female cohabitation is extremely important to sexual reproduction. Therefore, exploring the regulatory factors affecting the pairing of male and female schistosomes and their mechanisms of action is of great interest. Although the mechanisms underlying the action of GCP, a key molecule transferred between males and females, are not well understood, there is evidence for its participation in male-female interactions [2, 10–13, 23, 24]. In the present study, SjGCP was constructed as a bait protein, which was applied in a Y2H system. A Y2H cDNA library of *S. japonicum* at 18 days post-infection was also successfully constructed and the screening of proteins that interacted with SjGCP was performed using a yeast mating procedure. A total of 45 unique gene sequences were identified within 131 sequenced prey plasmids. Eventually, 24 proteins were found to interact with SjGCP after verification through yeast mating to exclude false positives. The interactions between two of them and SjGCP were then verified by co-immunoprecipitation.

Yeast two-hybrid technology has been widely used in recent years as a technical platform for discovering and studying the interactions between proteins in living cells. Co-immunoprecipitation is a powerful and simple method for detecting protein-protein interactions, and as a form of protein affinity chromatography, co-immunoprecipitation has sufficient sensitivity to detect weak interactions and provide a reliable means of identifying protein-protein interactions when used in combination with a Y2H system. In this study, among the 24 proteins that interacted with SjGCP in the yeast system, the interactions between SjGCP and SjLGL (SJCHGC06122) and SjCol-V(SJCHGC09290) were verified using co-immunoprecipitation. These experiments indicated that the interactive proteins screened out in this study were reliable.

Among the 24 interactive proteins, four (SJCHGC09290, SJCHGC05265, SJCHGC09378 and SJCHGC00545) belonged to the collagen family of proteins and were constituents of the extracellular matrix structure. SJCHGC04226 has procollagen-lysine 5-dioxygenase activity, SJCHGC05668 is a membrane-associated protein, SJCHGC00472 is an enolase, SJCHGC00694 is an actin, SJCHGC06072 is twinfilin, an actin-binding protein, SJCHGC00443 is a cytoplasmic fatty acid-binding protein (FABP), SJCHGC06074 is a UDP-glucose 4-epimerase, and SJCHGC06122 is a lethal giant larvae homolog protein (LGL). These proteins were found at the tegument of *S. japonicum* [42–45]. SJCHGC09129 (heat shock protein HSP60) and SJCHGC05222 (ubiquitin-conjugating enzyme E) are intra-cellular chaperones for other proteins, and they can covalently attach to other cellular proteins. These proteins have the basis for interacting with SjGCP proteins either at a specific location or while functioning. Studies have shown that some interacting SjGCP molecules such as SjFABP, SjLGL, and SjColV affect *S. japonicum* growth, development, and embryonic activity [44, 46].

In this study, the Y2H system was used to select proteins that interact with SjGCP, and the results indicated that the findings were reliable. Four proteins that were found to interact with SjGCP remain unknown or unannotated, even though genome sequencing and functional analysis of *S. japonicum* started in 2009 [47]. As the functions of these unannotated proteins are further elucidated, the mechanisms imparted by SjGCP on the growth and development of female *S. japonicum* worms will gradually be revealed, but the effects of Schistosoma males on females remain unclear and require further study.

Abbreviations

S. japonicum: *Schistosoma japonicum*; GCP: gynecophoral canal protein; *Sm*: *Schistosoma mansoni*; Y2H: yeast two hybrid; TGF: transforming growth factor; PBS: phosphate-buffered saline; HSP: heat shock protein; LGL: lethal giant larvae; FABP: fatty acid-binding protein; ColV: collagen alpha 1 (V).

Declarations

Ethics approval and consent to participate

Not applicable

Consent to publish

The author confirms: the work described has not been published before; it is not under consideration for publication elsewhere; its publication has been approved by all co-authors; its publication has been approved by the responsible authorities at the institution where the work is carried out.

Availability of data and materials

All data generated or analyzed during this study are included in this article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

YQR ML: overall experimental design and execution of all laboratory experiments, writing of the manuscript, interpretation of all data; PPL, QJW: participated in the retesting the protein interactions by yeast mating; YLS, YXY: participated and helped in the retesting the protein interactions by co-immunoprecipitation; LXZ, YMJ: obtained financial support, conceived and designed the study and analyzed the data.

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References

1. WHO. Schistosomiasis. Geneva: World Health Organization; 2018. <https://www.who.int/en/news-room/fact-sheets/detail/schistosomiasis>.
2. 24.LoVerde PT, Niles EG, Osman A, Wu W (2004) Schistosoma mansoni male female interactions. Can J Zool 82:357-374
3. Steinauer ML (2009) The sex lives of parasites: investigating the mating system and mechanisms of sexual selection of the human pathogen Schistosoma mansoni. Int J Parasitol 39(10):1157-1163

4. Kunz W (2001) Schistosome male-female interaction: induction of germ-cell differentiation. *Trends in parasitology* 17:227-231
5. LoVerde PT (2002) Presidential address. Sex and schistosomes: an interesting biological interplay with control implications. *Journal of Parasitology* 88:3-13
6. Basch PF, Basch N (1984) Intergeneric reproductive stimulation and parthenogenesis in *Schistosoma mansoni*. *Parasitology* 89:369-376
7. Popiel I, Cioli D, Erasmus DA (1984) The morphology and reproductive status of female *Schistosoma mansoni* following separation from male worms. *Int J Parasitol* 14:183-190
8. Popiel I (1986) Male-stimulated female maturation in *Schistosoma*: A review. *J Chem Ecol* 12:1745-1754
9. Chen MG (1991) Relative distribution of *Schistosoma japonicum* eggs in the intestine of man: a subject of inconsistency. *Acta Trop* 48(3):163-171
10. Lu Z, Spänig S, Weth O, Grevelding CG (2019) Males, the Wrongly Neglected Partners of the Biologically Unprecedented Male-Female Interaction of Schistosomes. *Front Genet* 10:796
11. Aronstein WS, Strand M (1985) A glycoprotein antigen of *Schistosoma mansoni* expressed on the gynecophoral canal of mature male worms. *Am J Trop Med Hyg* 34:508-512
12. Gupta BC, Basch PF (1987) Evidence for transfer of a glycoprotein from male to female schistosoma mansoni during pairing. *J Parasitol* 73 (3):674-675
13. Bostic JR, Strand M (1996) Molecular cloning of a *Schistosoma mansoni* protein expressed in the gynecophoral canal of male worms. *Mol Biochem Parasitol* 79(1):79-89
14. Elkins T, Zinn K, McAllister L, Hoffmann FM, Goodman CS (1990) Genetic analysis of a *Drosophila* neural cell-adhesion molecule: interaction of fasciclin-I and Abelson tyrosine kinase mutations. *Cell* 60:565-575
15. McAllister L, Goodman CS, Zinn K (1992) Dynamic expression of the cell adhesion molecule fasciclin-I during embryonic development in *Drosophila*. *Development* 115:267-276
16. McAllister L, Rehm EJ, Goodman CS, Zinn K (1992) Alternative splicing of micro-exons creates multiple forms of the insect cell-adhesion molecule fasciclin-I. *J Neurosci* 12:895-905
17. Hortsch M, Goodman CS (1990) *Drosophila* fasciclin I, a neural cell adhesion molecule, has a phosphatidylinositol lipidmembrane anchor that is developmentally regulated. *J Biol Chem* 265:15104-15109.
18. Zinn K, McAllister L, Goodman CS (1988) Sequence analysis and neuronal expression of fasciclin I in the grasshopper and *Drosophila*. *Cell* 53:577-587
19. Clout NJ, Tisi D, Hohenester E (2003) Novel fold revealed by the structure of a FAS1 domain pair from the insect cell adhesion molecule fasciclin I. *Structure* 11(2):197-203
20. Ahn CS, Kim JG, Bae YA, Kim SH, Shin JH, Yang Y, Kang I, Kong Y (2017) Fasciclin-calcareous corpuscle binary complex mediated protein-protein interactions in *Taenia solium* metacestode. *Parasit Vectors* 10(1):438

21. Horiuchi K, Amizuka N, Takeshita S, Takamatsu H, Katsuura M, Ozawa H, Toyama Y, Bonewald LF, Kudo A (1999) Identification and characterization of a novel protein, periostin, with restricted expression to periosteum and periodontal ligament and increased expression by transforming growth factor beta. *J Bone Miner Res* 14:1239-1249
22. Kim JE, Jeong HW, Nam JO, Lee BH, Choi JY, Park RW, Park JY, Kim IN (2002) Identification of motifs in the fasciclin domains of the transforming growth factor- β -induced matrix protein β ig-h3 that interact with the α v β 5 integrin. *J Biol Chem* 277:46159-46165
23. Osman A, Niles EG, Verjovski-Almeida S, LoVerde PT (2006) Schistosoma mansoni TGF-beta receptor II: role in host ligand-induced regulation of a schistosome target gene. *PLoS Pathog* 2 (6):e54
24. Cheng GF, Fu ZQ, Lin JJ, Shi Y, Zhou YC, Jin YX, Cai YM (2009). In vitro and in vivo evaluation of small interference RNA-mediated gynaecophoral canal protein silencing in Schistosoma japonicum. *J Gene Med* 11 (5):412–421
25. Lajoie JM, Cho YK, Frost D, Bremner S, Li L, Shusta EV (2019) A yeast display immunoprecipitation screen for targeted discovery of antibodies against membrane protein complexes. *Protein Eng Des Sel* 32(5):219-230
26. Zybaïlov BL, Byrd AK, Glazko GV, Rahmatallah Y, Raney KD (2016) Protein-protein interaction analysis for functional characterization of helicases. *Methods* 108:56-64
27. Chien CT, Bartel PL, Sternglanz R, Fields S (1991) The two-hybrid system: a method to identify and clone genes for proteins that interact with a protein of interest. *Proc Natl Acad Sci* 88:9578–9582
28. Fields S, Song O (1989) A novel genetic system to detect protein–protein interactions. *Nature* 340:245–246.
29. Smithers R, Terry RJ (1965) The infection of laboratory hosts with cercariae of schistosoma mansoni and the recovery of the adult worms. *Parasitology* 55:695-700
30. <http://www.clontech.com/images/pt/PT3247-1.PDF>
31. Wu QJ, Jin YM, Li M, Liu JM, Wang XB, Shi HY, Lin JJ (2010) Construction of DNABD Vector with SjGCP for yeast two hybrid system. *Chinese Journal of Animal Infectious Diseases* 18(3): 51- 55
32. Colley DG, Bustinduy AL, Secor WE, King CH (2014) Human schistosomiasis. *Lancet* 383(9936):2253–2264
33. Zhou XN, Wang LY, Chen MG, Wu XH, Jiang QW, Chen XY, Zheng J, Jürg U (2005) The public health significance and control of schistosomiasis in China—then and now. *Acta Trop* 96(2–3):97–105
34. Shen J, Lai DH, Wilson RA, Chen YF, Wang LF, Yu ZL, Li MY, He P, Hide G, Sun X (2017) Nitric oxide blocks the development of the human parasite Schistosoma japonicum. *Proc Natl Acad Sci* 114:10214–10219
35. Chen LL, Rekosh DM, LoVerde PT (1992) Schistosoma mansoni p48 eggshell protein gene: Characterization, developmentally regulated expression and comparison to the p14 eggshell protein gene. *Mol Biochem Parasitol* 52:39–52

36. Fitzpatrick JM, Hoffmann KF (2006) Dioecious *Schistosoma mansoni* express divergent gene repertoires regulated by pairing. *Int J Parasitol* 36:1081-1089
37. Grevelding CG, Sommer G, Kunz W (1997) Female-specific gene expression in *Schistosoma mansoni* is regulated by pairing. *Parasitology* 115:635-640
38. LoVerde P T, Rekosh D M, Bobek L A (1989) Developmentally regulated gene expression in *Schistosoma*. *Exp Parasitol* 68 (1):116-120
39. Menrath M, Michel A, Kunz W (1995) A female-specific cDNA sequence of *Schistosoma mansoni* encoding a mucin-like protein that is expressed in the epithelial cells of the reproductive duct. *Parasitology* 111:477-483
40. Schussler P, Grevelding CG, Kunz W (1997) Identification of Ras, MAP kinases, and a GAP protein in *Schistosoma mansoni* by immunoblotting and their putative involvement in male–female interaction. *Parasitology* 115:629-634
41. Siegel DA, Tracy JW (1989) *Schistosoma mansoni*: Influence of the female parasite on glutathione biosynthesis in the male. *Exp Parasitol* 69:116-124
42. Zhang M, Hong Y, Han YH, Han HX, Peng JB, Qiu CH, Yang JM, Lu K, Fu ZQ, Lin JJ (2013) Proteomic analysis of tegument-exposed proteins of female and male *Schistosoma japonicum* worms. *J Proteome Res* 12(11):5260-5270
43. Liu PP, Shi YL, Yang YX, Cao YF, Shi YL, Li H, Liu JM, Lin JJ, Jin YM (2012) *Schistosoma Japonicum* UDP-Glucose 4-Epimerase Protein Is Located on the Tegument and Induces Moderate Protection against Challenge Infection. *PLoS One* 7(7):e42050
44. Cao YF, Shi YL, Qiao HB, Yang YX, Liu JM, Shi YL, Lin JJ, Zhu G, Jin YM (2014) Distribution of lethal giant larvae (Lgl) protein in the tegument and negative impact of siRNA-based gene silencing on worm surface structure and egg hatching in *Schistosoma japonicum*, *Parasitol Res* 113(1):1-9
45. Liu F, Cui SJ, Hu W, Feng Z, Wang ZQ, Han ZG (2009) Excretory/secretory proteome of the adult developmental stage of human blood fluke, *Schistosoma japonicum*. *Mol Cell Proteomics* 1236-51
46. Yang YX , Jin YM, Liu PP, Shi YL, Cao YF, Liu JM, Shi YJ, Li H, Lin JJ (2012) RNAi silencing of type V collagen in *Schistosoma japonicum* affects parasite morphology, spawning, and hatching. *Parasitol Res* 111(3):1251-1257
47. Zhou Y , Zheng HJ, Chen X, Zhang L, Wang K, Guo J, Huang Z, Zhang B, Huang W, Jin K, Dou TH, Hasegawa M, Wang L, Zhang Y, Zhou J, Tao L, Cao ZW, Li YX, Vinar T, Brejova B, Brown D, Li M, Miller DJ, Blair D, Zhong Y, Chen Z, Liu F, Hu W, Wang ZQ, Zhang QH, Song HD, Chen SJ, Xu XN, Xu B, Ju Z, Cheng Y, Brindley PJ, McManus DP, Feng Z, Han ZG, Lu G, Ren SX, Wang YZ, Gu WY, Kang H, Chen J, Chen XY, Chen ST, Wang LJ, Yan J, Wang BY, Lv XY, Jin L, Wang BF, Pu SY, Zhang XL, Zhang W, Hu QP, Zhu GF, Wang J, Yu J, Wang J, Yang HM, Ning ZM, Beriman M, Wei CL, Ruan YJ, Zhao GP, Wang SY (2009) The *Schistosoma japonicum* genome reveals features of host-parasite interplay. *Nature* 460:345–351

Figures

Figure 1

Determination of the library quality using PCR. Lane M: DL2000 DNA marker; Lane 1–24: PCR products of randomly picked library clones

Figure 2

Detection of the library representative. Lane M: DL2000 DNA markers 1–6: six specific genes of *S. japonicum*: 1) SjCyclophilin C, 2) SjMagonashi, 3) SjGCP, 4) SjCyclophilin B, 5) SjZF1, and 6) Sj32

Figure 3

Retesting the interaction in yeast cells. Yeast AH109 containing pGADT7-SjLGL plasmids were mated separately with Y187 containing pGBKT7-SjGCP, pGBKT7-BD, and pGBKT7-Lamin C. Y187 containing pGBKT7-SjGCP were mated separately with AH109 containing pGADT7-AD and pGADT7-Lamin C. Clones from SD/-Leu/-Trp plates were streaked on SQDO/X- α -Gal tetra-deficient plates. The interaction between RecT and p53 was set up as a positive control.

Figure 4

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Retesting the interactions in mammalian cells by co-immunoprecipitation. (A) Construction of p3xFLAG-CMV-GCP and pCMV-Myc-LGL plasmids. Lane M: DL7000 DNA marker; Lane 1: the p3xFLAG-CMV-GCP plasmid digested by HindIII and SalI; Lane 2: the pCMV-Myc-LGL plasmid digested by SfiI and XhoI. (B) HEK293 cells were co-transfected with the expression plasmids for Myc-tagged LGL and FLAG-tagged GCP. The resulting input and immunoprecipitation samples (anti-Myc or anti-Flag) were subjected to SDS-page electrophoresis, and protein expression was detected using Myc and Flag antibodies.

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