Identification of HeLa cell proteins that interact with *Chlamydia trachomatis* glycogen synthase using yeast two-hybrid assays

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Abstract. *Chlamydia trachomatis* (*C. trachomatis*) is the leading cause of bacterial sexually transmitted diseases and infectious diseases that cause blindness. The pathophysiology of chlamydial infections is poorly understood, but secreted proteins have emerged as key virulence factors. *C. trachomatis* glycogen synthase (GlgA) is a chlamydial secretory protein, which localizes in the lumen of chlamydial inclusion bodies and the cytosol of host cells. In order to improve understanding of the roles of GlgA in chlamydial pathogenesis, four proteins that interact with GlgA, *Homo sapiens* CXXC finger protein 1, prohibitin (PHB), gelsolin-like actin-capping protein and apolipoprotein A-I binding protein were identified using yeast two-hybrid assays. The functions of these proteins are complex, and preliminary results suggested that PHB interacts with GlgA. However, further studies are required to determine the specific interactions of these proteins with GlgA. The findings of the present study may provide a direction and foundation for future studies focusing on the mechanism of GlgA in *C. trachomatis* infection.

Introduction

Chlamydia is a genus of gram-negative obligate intracellular bacteria consisting of nine recognized species; each species exhibits specific tissue tropism and disease pathology (1). The global impact of *Chlamydia trachomatis* (*C. trachomatis*) is considerable, and of significant medical concern in humans (2). *C. trachomatis* exists as 19 serovars; serovars A-C are responsible for trachoma, the leading infectious cause of blindness worldwide (3). Serovars D-K primarily infect the genital mucosae, causing numerous commonly-diagnosed sexually transmitted diseases, including hydrosalpinx, a laparoscope-detectable marker of tubal factor infertility (4). Moreover, *C. trachomatis* is also a major risk factor in the transmission of human immunodeficiency virus (5). To the best of our knowledge, no study to date has determined why *C. trachomatis* can cause infectious blindness, or how infection of the lower genital tract can result in tubal fibrosis and hydrosalpinx. Therefore, the aim of the present study was to determine the molecular mechanisms of *C. trachomatis* pathogenicity, and to guide the design of live-attenuated vaccine strains for the prevention of chlamydial diseases.

As with all other chlamydia, *C. trachomatis* possesses a unique intracellular growth cycle with a distinct biphasic developmental cycle, alternating between an infectious elementary body (EB) and a replicating, metabolically-active reticulate body (RB) (6). EBs differentiate into RBs within a non-acidified vacuole, the chlamydial inclusion (7). At ~18 h post-infection, the generated progeny differentiate back into EBs. Later in the developmental cycle, EBs are released from the host cell to initiate a new cycle of infection (2).

Host inflammatory responses triggered by chlamydial intracellular survival and replication contribute to chlamydia-induced pathologies; secretory proteins, including chlamydia protease-like activity factor (CPAF), have been hypothesized to play important roles in this process (8). CPAF, secreted into the cytosol of *C. trachomatis*-infected cells, is involved in the immune evasion mechanism that may block NF-κB p65 nuclear translocation, resulting in decreased interferon-β and pro-inflammatory cytokine synthesis (9). Despite extensive research into CPAF, the mechanism of *C. trachomatis*-associated trachoma and infertility following infection remains unknown. Asmost chlamydia-infected host cells secrete chlamydial proteins, other secreted proteins are also likely to notably contribute tothe pathogenicity of chlamydial infections in humans.

In a previous study, in fixed *C. trachomatis*-infected HeLa cells analyzed with antibodies against chlamydia glycogen

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metabolism-related enzymes, only *C. trachomatis* glycogen synthase (GlgA) was found to be secreted into the host cell cytosol (10). It was first revealed to be associated with chlamydial inclusion bodies at 12 h post-infection, and secretion into the cytosol was detectable at ~24 h post-infection. However, since glycogen was only monitored in the inclusion bodies, and not the cytosol, it is unclear whether GlgA secretion into the host cell cytosol is necessary for the induction of chlamydial diseases.

GlgA expression is dependent on a cryptic plasmid; removal of this plasmid results in the loss of GlgA expression and attenuated pathogenicity in both *C. trachomatis* serovar A and *C. muridarum* (11). These findings indicate that GlgA may play an essential role in chlamydial pathogenesis.

The yeast two-hybrid system enables the *in vivo* detection of interacting proteins in order to reveal the biological roles of a known protein (12). Following a series of optimizations and development by Fields and Song (13), the yeast two-hybrid system was considered to bea classical method ofidentifying and studying protein-protein interactions. In a recent studyusing the yeast two-hybrid system, the *C. trachomatis* inclusion membrane protein MrcAwas found tointeract with inositol 1,4,5-trisphosphate receptor type 3 to regulate extrusion formation (14). Thus, due to its inexpensive and time-saving nature, the yeast two-hybrid system is a powerful method for the analysis of protein-protein interactions.

In the present study, the yeast two-hybrid system was used to identify proteins that interact with GlgA. This strategy involved screening 13 potential clones, which following cDNA identification, were confirmed via rotary validation and co-immunoprecipitation. The results indicated that prohibitin (PHB) interacts with GlgA, which may provide novel insight into the understanding of GlgA in chlamydial biology and pathogenesis.

Materials and methods

Bait plasmid construction. The Matchmaker two-hybrid system (Clontech Laboratories, Inc.) was used to confirm the potential interaction partners of GlgA (CT798). The gene sequence of CT798 (WP-100139618) was obtain from the National Centre for Biotechnology Information database (https://www.ncbi.nlm.nih.gov/) and amplified by PCR using the following primers, which contained *Sfi*I sites at both ends: CT798 forward, 5'-AAGGCC ATTACGGCCATGAAAATTATTCACACAGCTATCG-3' and reverse, 5'-CCGGCCGAGGCGGCCTTGTTTATAAATTTC TAAATATTTATTGGC-3'.

PCR was performed under the following conditions: Initial denaturation at 98˚C for 5 min, amplification (30 cycles) at 98˚C for 30 sec, 55˚C for 30 sec and 72˚C for 42 sec, and afinal extension at 72˚C for 5 min (Qiagen). The FastPfu DNA Polymerase was used (Beijing Transfen Biotech Co., Ltd.). The orientation and authenticity of the plasmid sequence was confirmed by sequencing (Sangon Biotech Co., Ltd.) and restriction endonuclease digestion by 8% SfiI digestion (Fermentas) at 50˚C for 24 h.

Recombinant plasmid confirmation. The CT798 plasmid was cloned into the GAL4 binding domain of the pGBKT7 vector (Hitech Bio-Technology Co., Ltd). CT798 plasmids were treated with *Sfi*I in a 25-μl reaction system including 20 μl CT798, 2 μl *Sfi*I restriction enzyme, 2.5 μl 10X buffer and 0.5 μl sterile water. pGBKT7 was treated with *Sfi*I in a 20-μl reaction system including 10 μl CT798, 1 μl *Sfi*I restriction enzyme, 2 μl 10X buffer and 7 μl sterile water. The digested reaction mixtures were then combined and incubated at 16˚C for 1 h, consisting of 5 μl Ligation High Buffer (Toyobo Life Sciences), 5 μl CT798/*Sfi*I and 0.5 μl pGBKT7/*Sfi*I. The recombinant plasmid wasthenused to transform TOP10 *Escherichia coli*. *Escherichia coli* was supplied by American Type Culture Collection and cultured in Luria-Bertani medium (1% NaCl; 1% Polypeptone; 0.5% Yeast extract) in a humidified incubator at 37˚C with 5% CO_2. Then, it was inoculated onto Luria-Bertani medium plates (1% NaCl; 1% Polypeptone; 0.5% Yeast extract; 2% agar) containing 50 μg/ml kanamycin (LB-Kan^{r+}) overnight at 37˚C. A total of six bacterial colonies were selected and further cultured at 16˚C overnight with agitation (250 x g). The plasmids of the cultured bacteria were extracted using a Plasmid Miniprep kit (Qiagen, Inc.) and verified by sequencing.

Agarose gel electrophoresis. Agarose gel electrophoresis is a standard method for identification DNA fragments (15). In the present study, 0.7% agarose gel was used. Then, 0.14 g agarose and 20 ml 1X Tris-acetate-EDTA buffer in a flask were heated in a microwave for 5 min at 100˚C. Then, 2 μl ethidium bromide was added (Thermo Fisher Scientific, Inc.), and poured onto a taped plate with casting combs. Then, 2 μl DNA sample from *Escherichia coli* was added to the 5X agarose gel, and underwent electrophoresis at 120 mA for 40 min at 25˚C until separation had been achieved. The DNA fragments were visualized using a long wave UV light box.

Self-activation test. pGADT7, pGBKT7-p53, pGBKT7-laminC and the pGADT7-170297-LUcDNA plasmid genome were provided from Hitech Bio-Technology Co., Ltd. The pGADT7-170297-LUcDNA plasmid genome was referred to as 'pGADT7-largeT'. As a positive control, pGBKT7-p53 and pGADT7-largeT were used to transform AH109 yeast cells. As a negative control, AH109 cells were also transformed using pGBKT7-lamin C with pGADT7-large T. AH109 were purchased from the American Type Culture Collection and cultured in yeast peptone dextrose adenine (YPDA; 1% Yeast extract; 2% Tryptone; 2% Glucose; 0.02% Adenine) with 5% CO_2. Synthetic defined (SD)/-tryptophan (Trp), SD/-leucine (Leu), SD/-Trp/-Leu, SD/-Trp/-Leu/-histidine (His) and SD/-Trp/-Leu/-His/-adenine (Ade) media were purchased from Clontech Laboratories, Inc.

To test whether the prey plasmid was able to auto-activate the reporter genes, pGADT7 and pGBKT7-CT798 were used to co-transform AH109 cells according to the lithium acetate transformation method (16). The cells were then cultured in SD medium lacking various amino acids (SD/-Trp/-Leu) at 30˚C for 4 days. The isolated yeast clones were tested for the activation of the third reporter gene, including imidazoleglycerol-phosphate dehydratase (HIS3), phosphoribosylaminoimidazole carboxylase (ADE2) and LacZ.

Yeast two-hybrid screening. The pGADT7-170297-LUcDNA plasmid genome from HeLa cells was used for the yeast

two-hybrid screen (17). AH109 yeast cells containing the pGBKT7-CT798 plasmid were plated and selected using SD agar plates without tryptophan. The positive clones were inoculated in 50 ml SD/-Trp medium and cultured at 30˚C (225 x g) for 18 h. The cells were then transferred to 500 ml YPDA medium and cultured at 30˚C (agitation at 225 x g) for 4 h until OD_{600}=0.6. Next, the cultured cells were centrifuged at 4,000 x g for 5 min at room temperature; the supernatant was discarded and the pellet was resuspended in 0.1 MLiAc of 20 ml. The cells were then centrifuged once more under the same conditions and suspended in 10 ml LiAc (0.1 M). Then, 9.6 ml 50% PEG3350, 1.44 ml LiAc, 300 μl single-stranded DNA and 25 μg cDNA library plasmid were added, and the cells were cultured at 30˚C for 30 min. The mixture was heated in a water bath at 42˚C for 25 min, followed by resuscitation at 30˚C for 1 h. The cell pellet was resuspended in 8 ml sterile water following centrifugation at 4,000 x g for 5 min at room temperature. The cells were cultured on SD/-Leu/-Trp/-His and 3-amino-1,2,4-triazole (3AT) plates with 200 μl solution/plate, for a total of 40 plates. In order to determine library transformation efficiency, 20 μl cell cultures from 8 ml solution were diluted 10-fold by gradient and cultured on SD/-Leu/-Trp plates. The cells were cultured for 3-4 days at 30˚C, with observation ofthe conversion results and recording of conversion efficiency. The following calculations were used: Total number of transformants = (n_1/20+n_2/2+n_3/0.2)x1/3x8,000). Where n_1, the number of colonies growing on the plate after 10-fold dilution; n_2, the number of colonies growing on the plate after 100-fold dilution; n_3, the number of colonies growing on the plate after 1,000-fold dilution. Transformation efficiency = Total number of transformants/25 μg cDNA plasmid.

Positive clone verification. After culturing on SD/-Leu/-Trp/-His and 3AT plates, the positive clones were plated onto SD/-Leu/-Trp plates. To select for clones which potentially interacted with CT798, the clones were inoculated onto SD/-Leu/-Trp/-His+3AT and SD/-Leu/-Trp/-Ade plates to assess the transcriptional activation of the HIS3 and ADE2 reporter genes, and resuspended in sterile water to detect LacZ expression. Concurrently, positive and negative control transformation experiments were conducted, for the purpose of selecting true positive colonies. The positive clone plasmids were subsequently extracted using the Plasmid Miniprep kit. Following sequencing, a BLAST search was conducted using GenBank (National Center for Biotechnology Information) to determine the associated genes.

Rotary validation. The selected clones were used to transform AH109 yeast cells containing pGBKT7-CT798 as aforementioned, and these cells were cultured on SD/-Leu/-Trp plates. Positive clones were transferred onto SD/-Leu/-Trp/-His/-Ade+3AT and SD/-Leu/-Trp/-Ade plates to determine the transcriptional activation of the HIS3 and ADE2 reporter genes, and resuspended in sterile water to detect LacZ expression.

Co-immunoprecipitation. HeLa cells were provided from the University of South of China and cultured in complete DMEM (Gibico; Thermo Fisher Scientific, Inc), containing 10% FBS (Gibico; Thermo Fisher Scientific, Inc.) in a humidified incubator at 37˚C with 5% CO_2. HeLa cells were infected with *C. trachomatis*. *C. trachomatis* was added into HeLa cell. At 48 h post-infection, the cells were washed twice with cold PBS, and lysed with RIPA buffer (Epizyme, Inc.) for 15 min at 4˚C. The lysate was pre-clarified by rotation for 10 min, centrifuged at 14,000 x g for 15 min (both 4˚C), and incubated with protein-A agarose beads. Cell-free lysates were incubated with a rabbit anti-PHB antibody (1:1,000; Cell Signaling Technology, Inc.; cat. no. ab75766.) or IgM antibody (1:1,000; Cell Signaling Technology, Inc.) at 4˚C overnight. Protein-A beads were added for 2 h at room temperature, and the lysates were subsequently washed three timeswith PBS. The proteins were eluted into 5X SDS-PAGE sample buffer, separated by 12% SDS-PAGE gel for immunoblotting.

Western blotting. HeLa cells were infected with *C. trachomatis* and harvested at 48 h post-infection. Cells were washed with cold PBS and lysed with RIPA buffer (Epizyme, Inc.) with protease inhibitor and phosphatase inhibitor. Then, 10 μl protein extracts and cell lysates (Input control) were separated by 12% SDS-PAGE gel and electrophoretically transferred to nitrocellulose membranes. After blocking in 5% non-fat dry milk with TBST (50 mM Tris-HCl; pH 7.4; 5.36 mM KCl; 274 mM NaCl; 0.1% Tween-20) for 2 h at room temperature, the membranes were probed with a mouse anti-CT798 antibody (1:1,000; The University of South China) overnight at 4˚C. Then, the membrane waswashed four times for 5 min in TBST and incubated with anti-mouse horseradish peroxidase-conjugated secondary antibody (1:3,000; Servicebio, Inc.; cat. no. GB23303) for 1 h at 37˚C Blots were detected by Western Blot system G: BOX Chemi XXX9 (Syngene International Ltd.).

Results

Construction of the pGBKT7-CT798 bait plasmid. CT798 is 53 kDa; the full length of CT798 (1,440 bp) was successfully amplified from *C. trachomatis* genomic DNA viaPCR, and analyzed using restriction enzyme digestion (Fig. 1A). The PCR products were subsequently cloned into the bait vector pGBKT7. The construct was transformed into *E. coli* and inoculated onto LB-kan^{r+} plates. Following incubation, six positive transformants were randomly picked and identified by PCR (Fig. 1B), and the results of recombinant plasmid sequencing were consistent with that of pGBKT7-CT798.

Validity of the bait plasmid pGBKT7-CT798. The AH109 yeast strain contains various nutritional reporter genes. These include: i) ADE2, coding for phosphoribosylaminoimidazole carboxylase, an important enzyme for adenine synthesis; ii) HIS3, encoding imidazoleglycerol-phosphate dehydratase, another key enzyme for adenine synthesis; and iii) LacZ, which encodes β-galactosidase enzymes. Therefore, AHI09 allows for the control of strong nutritional selection, whilst reducing the incidence of false positives. To determine the efficiency of the system, pGBKT7-CT798 and pGADT7 were used to transform AH109 cells, and pGBKT7-p53 and pGBKT7-laminC were used as the positive

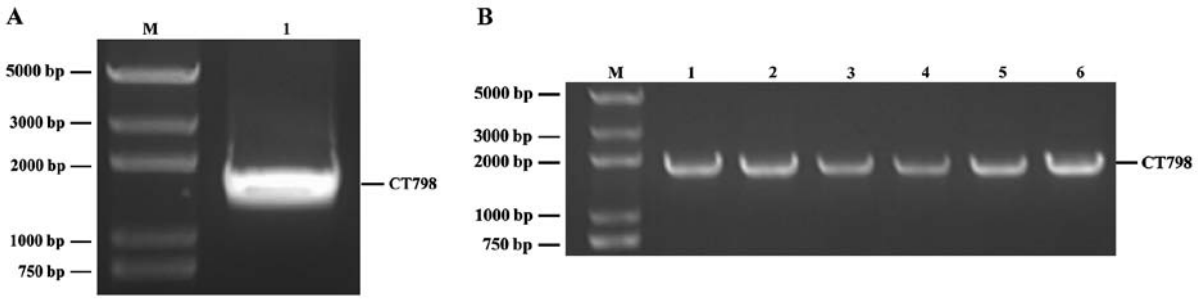

Figure 1. Construction of the bait plasmid. PCR products were separated on a 1% agarose gel, and the DNA was visualized using ethidium bromide staining. (A) Restriction enzyme digestion of pET28a-CT798. (B) CT798PCR product from different clones. CT798, *Chlamydia trachomatis* glycogen synthase (also known as GlgA).

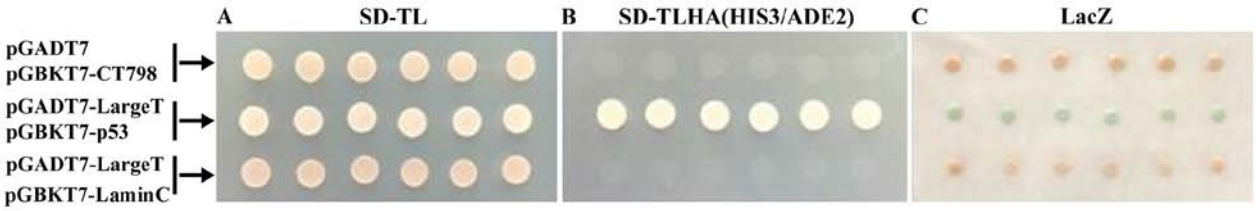

Figure 2. CT798 has no self-activation function. Growth of AH109 cells containing pGADT7/pGBKT7-CT798 and pGADKT7-Large/pGBKT7-P53 on plates with different nutrient deficiencies. pGADKT7-Large/pGBKT7-P53 was used as the positive control, and pGADT7-LargeT/pGBKT7-LaminC as the negative control. (A) Diploid growth on SD-TL plates. Only the positive control can (B) be grown on SD-TLHA plates and (C) express LacZ. CT798, *Chlamydia trachomatis* glycogen synthase (also known as GlgA); SD, synthetic defined; -TLHA, -tryptophan/-leucine/-histidine/-adenine; HIS3, imidazoleglycerol-phosphate dehydratase; ADE2, phosphoribosylaminoimidazole carboxylase.

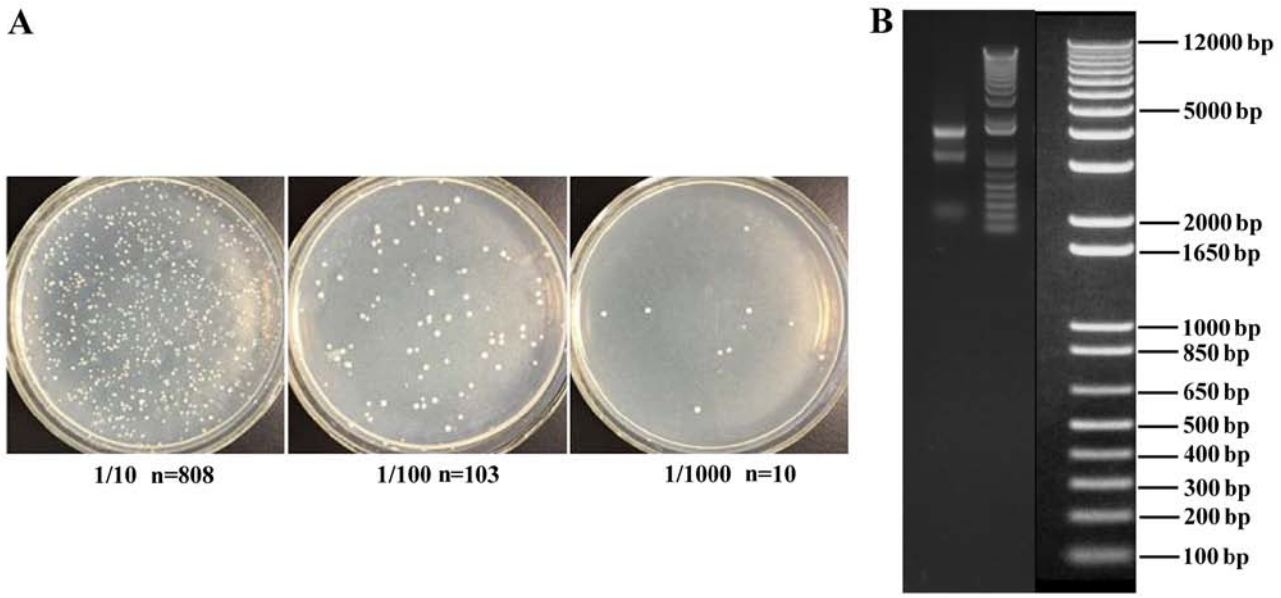

Figure 3. Transformation efficiency. (A) A total of 25 μg cDNA library plasmid was used to transform AH109 yeast cells containing pGBKT7-CT798. The cell pellet was resuspended in 8 ml sterile water, serially diluted, and 20 μl was cultured on SD/-leucine/-tryptophan plates. Total number of transformants =(808/20) + (103/2) +(10/0.2) x 1/3x8,000=3.78x10^5. Transformation efficiency =3.78 x10^5/25 μg =1.51x10^4/μg. (B) Agarose gel electrophoresis of different clones.

and negative controls, respectively. AH109 cells were then cultured on SD/-Leu/-Trp plates, and six transformants were used to test for self-activation, including pointing-plate culturing on SD/-Leu/-Trp/-His/-Ade plates to test for the expression of HIS3 and ADE2, and a colorimetric assay to determine the expression of LacZ. Control strains both grow on the SD/-Leu/-Trp plate, but only the positive control cells are able to grow on the SD/-Leu/-Trp/-His/-Ade plate. In the present study, AH109 cells containing the pGBKT7/CT798 plasmid grew on the SD/-Leu/-Trp plate, but not on the SD/-Leu/-Trp/-His/-Ade plate. Furthermore, the results of the colorimetric assay were consistent with those of the negative control (Fig. 2). These results suggest that CT798 does not possess self-activation function in AH109 cells.

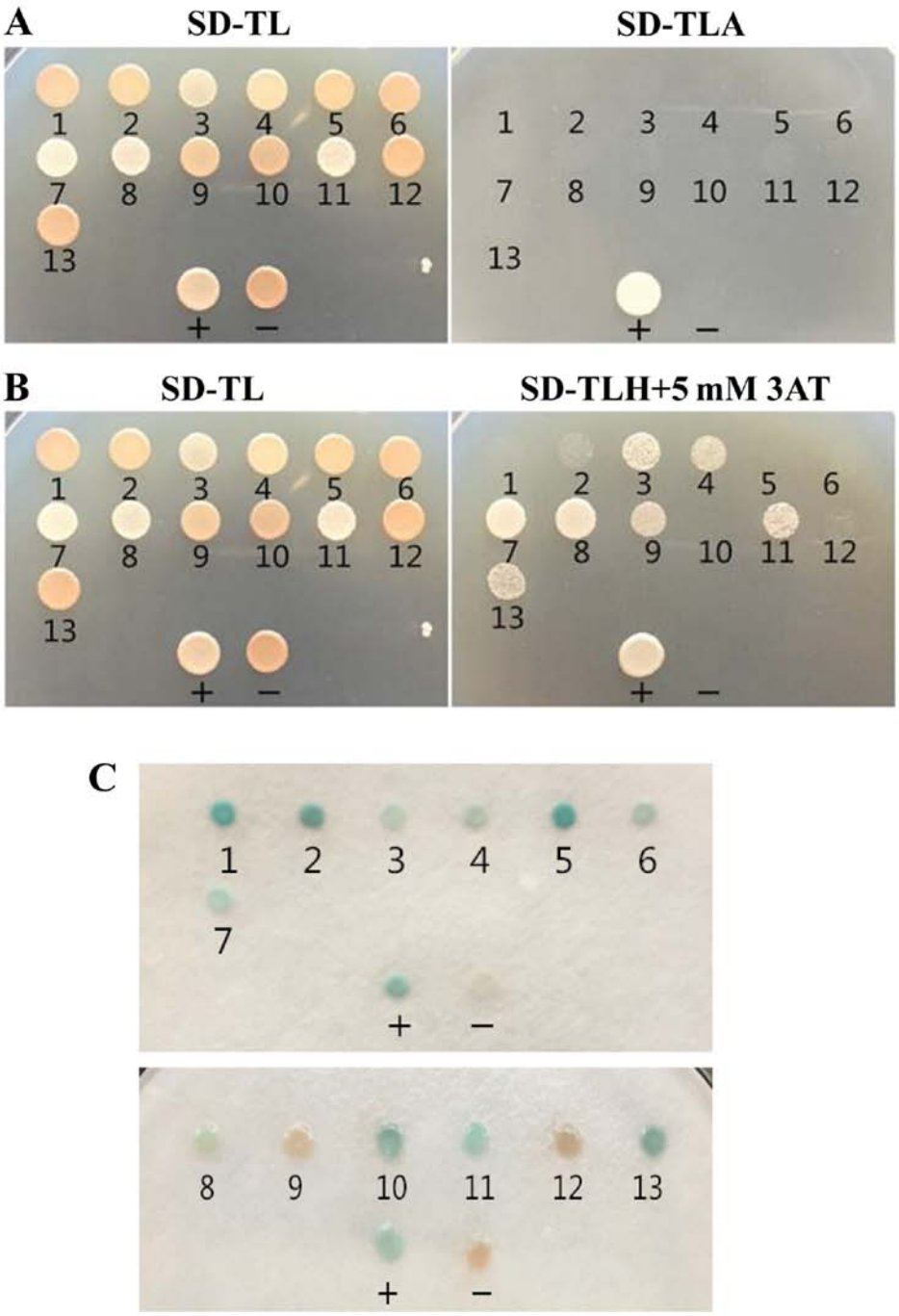

Figure 4. Yeast two-hybrid screening. Interactions identified by yeast-two hybrid analysis were verified by screening diploids grown on SD-TL plates, and in high stringency medium. (A) No interactions were detected on SD-TLA plates, resulting in blue yeast colonies. (B) A total of seven interactions were identified using SD-LTH +3AT plates, and (C) 11 interactions activated the LacZ reporter gene. SD, synthetic defined; -TLHA, -tryptophan/-leucine/-histidine/-adenine; 3AT, 3-amino-1,2,4-triazole.

cDNA library screening. pGADT7-170297-LUcDNA library used for screening was supplied by Hitech Bio-Technology Co., Ltd. Plasmids contained only pGBKT7-CT798 as bait for screening the pGADT7-170297-LUcDNA library. Library plasmids were transformed into AH109 cells containing bait plasmid and cultured on SD/-Leu/-Trp/-His and 3AT plates. AH109 cells were also cultured on SD/-Leu/-Trp plates (Fig. 3), and transformation efficiency (which is important for the success of the yeast two-hybrid system) was determined. The results revealed a total of $3.78x10^5$ transformants, indicating a transformation efficiency of $1.51x10^4/\mu g$. At the same time, the quality of the yeast two-hybrid library was assessed. Agarose gel electrophoresis of the PCR products indicated the average length of the insertion product to be ~1.5 kb, and the positive success rate of the library was 100%.

Identification of proteins interacting with the bait protein. In the present study, 13 positive clones were obtained from two rounds of selection, which were designated A1-13. These clones were not capable of growing on SD/-Leu/-Trp/-Ade plates, and seven grew on SD/-Leu/-Trp/-His and 3AT plates (Fig. 4). In addition, 11 of the 13 clones expressed LacZ.

Table I. Biological information of the seven positive clones.

Clone(s)	NCBI accession	NCBI description
A3	XM_017025718.1	PREDICTED: *Homo sapiens* CXXC finger protein 1, transcript variant X2, mRNA
A4, A9	XM_017024763.1	PREDICTED: *Homo sapiens* prohibitin, transcript variant X2, mRNA
A7, A8	NM_001256139.1	*Homo sapiens* capping actin protein, gelsolin-like, transcript variant 2, mRNA
A11, A13	AK298222.1	*Homo sapiens* cDNA FLJ56357 complete cds, highly similar to *Homo sapiens* apolipoprotein A-I binding protein, mRNA

NCBI, National Center for Biotechnology Information.

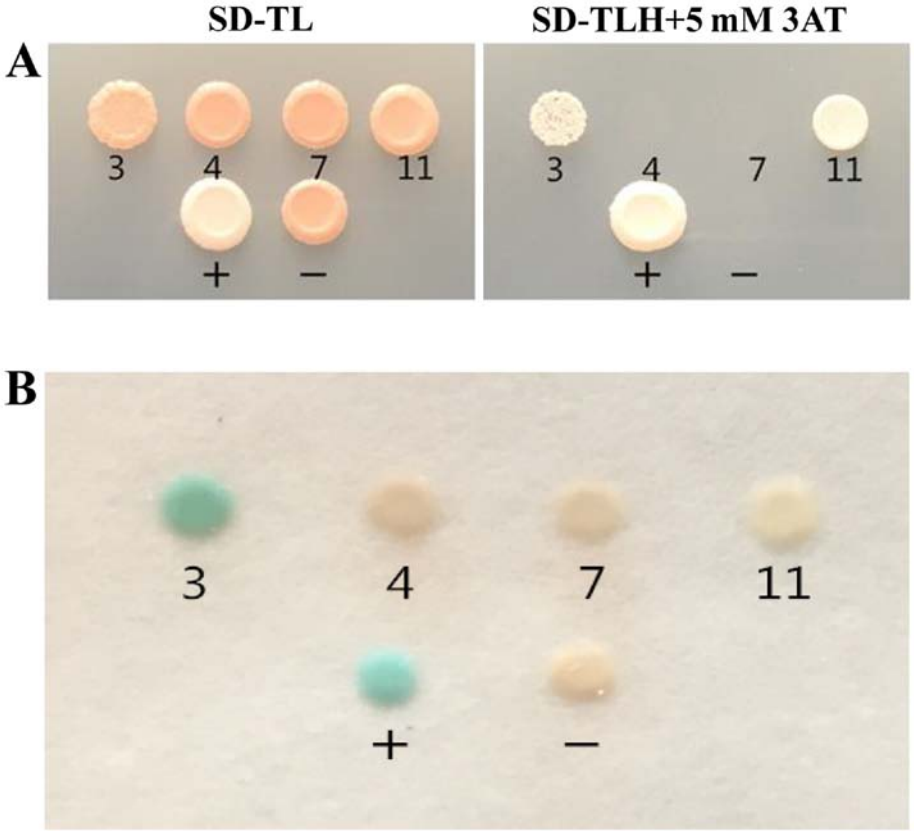

Figure 5. Rotary validation. After screening, clones for the four selected genes were used to transform into AH109 yeast cells containing pGBKT7-CT798. The diploids were cultured on SD-TL plates and in high stringency medium. (A) Clones A3 and A11 successfully grew on SD-TLH +3AT plates, and (B) clone A3 was able to active the LacZ gene. SD, synthetic defined; -TLH, -tryptophan/-leucine/-histidine; 3AT, 3-amino-1,2,4-triazole.

These results indicated that the HIS3 gene was activated by the seven positive clones, and further suggested that A3, A4, A7, A8, A9, A11 and A13 interacted with CT798 to a certain degree. The positive clones were subsequently sequenced and analyzed using BLAST software. The results indicated that the seven positive clones possessed a high degree of similarity to known genes encoding four different proteins (Table I). Finally, for rotary validation, the resulting four plasmids were used to transform AH109 cells containing the pGBKT7-CT798 plasmid (Fig. 5). The results revealed that clones A3 and A11 were able to active the HIS3 gene, and that only A3 activated the LacZ gene.

PHB interacts with CT798. Co-immunoprecipitation was performed to validate the interaction between CT798 and PHB (Fig. 6). CT798 was co-precipitated by anti-PHB from the lysates of cells infected with *C. trachomatis*, but not by precipitation with IgM. This suggested that PHB interacts with CT798.

Discussion

GlgA is a key enzyme involved in glycogen metabolism, which catalyzes the glucose moiety of UDP-glucose transferred to the non-reducing end of a linear α-1, 4-glucan primer. Previous

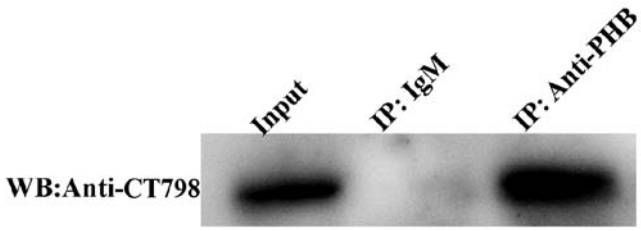

Figure 6. Interaction between CT798 and PHB. HeLa cells were infected with *C. trachomatis*, harvested 48 h post-infection, and the lysates were prepared for co-immunoprecipitation. Supernatants from the infected lysates were incubated with protein-A beads that had been pre-loaded with anti-PHB or the negative control antibody IgM. Immunoblots were probed with anti-CT798. CT798, *Chlamydia trachomatis* glycogen synthase; PHB, prohibitin; WB, western blotting.

studies have reported that the inactivation of GlgA not only inhibits glycogen synthesis, but also reduces bacterial stress resistance (18,19). It is possible that GlgA is associated with adaptive environmental survival, colonization and virulence. In addition, the average chlamydial genome size is ~1.0 Mb, only 1/4 of that of *E. coli* (20). Notably, the expression and secretion of GlgA is highly conserved among different serovars of *C. trachomatis* and *C. muridarum*, a separate, but related species (21). The importance of GlgA in chlamydial growth and development is well established (10). However, the effects of GlgA secretion into the host cell and its involvement in chlamydial metabolism (which may serve a role in the association of GlgA secretion with the inflammatory response) are yet to be investigated. Thus, screening for binding partners of GlgA using the yeast two-hybrid system will enhance current understanding of the role of GlgA in chlamydial biology and pathogenesis.

Yeast two-hybrid technology, an effective means of detecting unidentified protein-protein interactions, has allowed for further exploration of biological pathways and their regulation (22). In the present study, not only were positive and negative controls used, but promoters modulating HIS3, ADE2 and LacZ reporter gene expression in the AH109 yeast strain were also included to exclude false-positive results. Although the yeast two-hybrid assay is associated with high sensitivity and easy operation, it exhibits obvious drawbacks, and false positives are still easily obtained. Hence, other *in vitro* assays (such as immunoprecipitation) should be conducted to verify these results and improve data credibility.

To enhance the probability of identifying unambiguous interactions, stringent media were used, which lacked the amino acid histidine or the nucleic acid adenine (23). Growth-dependent selection enables the most effective identification of interaction partners. Therefore, only bait-prey interactions result in HIS3 or ADE2 reporter gene expression; LacZ expression provides a reference to reflect the strength of the interactions. The identified interaction proteins were *Homo sapiens* CXXC finger protein 1 (CFP1), PHB, gelsolin-like actin-capping protein (CAPG) and apolipoprotein A-I binding protein (ApoA-I).

CFP1, an unmethylated CpG-binding protein, is a component of the mammalian set1 histone methyltransferase complex, which is involved in histone methylation, regulating T-cell development and promoting cellular differentiation (24). Anti-chlamydial T cells may represent a double-edged sword, and may be responsible for initiating the pathological changes associated with chlamydial infection (25). Thus, T-cell secretion of GlgA and CFP1 may promote chlamydial persistence and immunopathology.

PHB is characterized as an anti-proliferative gene, and is widely distributed in different cellular compartments, such as the plasma membrane, nucleus and mitochondria (26). PHB family members possess two subunits, PHB1 and PHB2; PHB1 binds to the transcription factor p53, inducing p53-inducible gene 3 promoter binding and promoting p53-dependent cell apoptosis (27). PHB2 can translocate from the mitochondria to the nucleus during capsaicin-induced apoptosis (28). PHB1 and 2 are conserved mitochondrial proteins with diverse functions, which include roles in cell proliferation and mitochondrial integrity (29). As obligate intracellular pathogens, chlamydia have been proposed to be anti-apoptotic to complete their development cycle (30). Accordingly, PHB is hypothesized to shuttle between the mitochondria and the nucleus during the cell cycle, and GlgA is secreted into the cytosol at ~24 h post-infection (31). These findings indicate that at the beginning of the developmental cycle, the manipulation of GlgA by *C. trachomatis* may prevent premature release of inclusion bodies from the cell via its interaction with PHB.

The actin-binding protein CAPG was originally identified in the cytoplasm of alveolar macrophages (32). CAPG interacts with cytoplasmic and nuclear proteins that are closely associated with cell proliferation and motility (33). It promotes restructuring of the actin cytoskeleton by capping the fast-growing end of actin filaments in a Ca^{2+} and phosphatidylinositol 4,5-bisphosphate-dependent manner, but does not initiate actin filament severing (34). Furthermore, CAPG may be involved in mediating cross-talk between the actin cytoskeleton and the microtubule-based organelles that regulate cell division (35). *C. trachomatis* has evolved exclusion mechanisms of host-cell escape, through which membrane-bound inclusions are released from the host cell viaa process resembling exocytosis (36). This process is hypothesized to be dependent on cytoskeletal activities, including actin polymerization, as well as neural Wiskott-Aldrich syndrome protein, Rho GTPase and the myosin motor complex (37). The results of the present study demonstrated that CAPG may interact with GlgA to regulate extrusion formation, which facilitates evasion of the localized inflammatory response at the site of infection.

ApoA-I is the primary constituent of plasma high-density lipoprotein (HDL) and plays a central role in reverse cholesterol transport (RCT) (38). The process of RCT, which transports cellular cholesterol back to the liver, is considered to be one of the most important anti-atherogenic properties of HDL (39). Several experimental strategies aimed at overexpressing ApoA-I are associated with the anti-atherogenic effects of HDL (40). Besides upper respiratory infection, chlamydial challenge has also been associated with chronic inflammatory conditions, including atherosclerosis (41). The interaction between GlgA and ApoA-I has been speculated as a novel target for the treatment and prevention of severe long-term sequelae of chlamydial infections. As these interacting proteins are known to be associated with immunoregulation, apoptosis and tumor progression, it is possible that GlgA operates in a similar manner.

In the present study, four potential GlgA-interaction proteins were identified. However, three plasmids did not

pass rotary validation, which may be due to unsuccessful transformation of prey or bait plasmids into AH109 or weak protein-protein interactions. Here, full-length of PHB was found to interact with GlgA via co-immunoprecipitation. But unfortunately, the part of PHB that interacts with GlgA was not determined in this study, even though PHB1 and PHB2 belong to the PHB family. Thus, subsequent studies will investigate the interacting part of GlgA to further clarify the relation between *C. trachomatis* infections and GlgA secretion into the host cell cytosol. In summary, the yeast two-hybrid system was successfully exploited to screen for GlgA interaction partners, indicating further regulatory functions of GlgA.

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Availability of data and materials

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

Authors' contributions

ZS, YS, YL, XL, HC, HW and BP performed the experiments. ZS wrote the paper. CL designed the study, coordinated the data analysis, reviewed and edited the manuscript. All authors read and approved the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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