

Expression and functional characterization of a Rho-family small GTPase CDC42 from *Trichinella spiralis*

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Abstract A full-length cDNA encoding a Rho-family small GTPase gene *cdc42* of *Trichinella spiralis* was expressed in *E. coli*. The recombinant protein of TsCDC42 was purified and used to raise the polyclonal antibodies. The expression of TsCDC42 in different stages of parasite was investigated. The western blot showed that TsCDC42 was expressed in all stages of *T. spiralis*, including newborn larvae, muscle larvae and adult worms. The immuno-localization revealed that TsCDC42 was ubiquitously distributed in the newborn larvae, muscle larvae and adult worm. Cross-species RNAi was done by knockdown *Tscdc42* RNAi in *C. elegans*. The results revealed that endogenous expression level of CDC42 was decreased by knockdown *Tscdc42* RNAi in *C. elegans*, and this knockdown reduced the progeny of *C. elegans*. It suggested that *Tscdc42* might play the same roles in the early development of *T. spiralis*.

Introduction

Trichinella are important animal parasitic nematodes which can lead to serious zoonoses called trichinellosis. The adult worms of *Trichinella* parasitize in the intestine, and newborn

larvae migrate to the striated skeletal muscle via the blood system where they grow into infective muscle larvae and encapsulate within myofibrils until another host animal ingests them. Human trichinellosis outbreaks have been reported over the worldwide area (Akkoc et al. 2009; Calcagno et al. 2005; Dubinsky et al. 2001; Golab and Sadkowska-Todys 2006; Gong et al. 2008; Kaewpitoon et al. 2008; Kaewpitoon et al. 2006; Khumjui et al. 2008; Moller et al. 2005; Ozeretskovskaya et al. 2005; Pozio et al. 2006; Ribicich et al. 2005; Rodriguez de las Parras et al. 2004; Schellenberg et al. 2003; Tverdokhlebova et al. 2005). Trichinellosis is prevalent in many parts of China (Liu and Boireau 2002; Wang and Cui 2001; Wang et al. 2006). As one of the most common food-borne parasitic zoonoses pathogens, trichinellosis causes heavy economical losses as well as a threat to human health.

Rho family of small GTPase CDC42 acts as an intracellular signaling molecular switch to cycle between GTP-bound active state and GDP-bound inactive state. These molecules are activated by guanine nucleotide exchange factors (GEFs) and inactivated by GTPase-activating proteins (GAPs). GEFs promote the exchange of GDP for GTP on GTPase. GAPs inactivate GTPase via their intrinsic activity (Lundquist 2006). CDC42 can be activated to recognize downstream effectors and regulate a variety of cell functions including cell polarity, morphogenesis and motile properties (Kim 2000). CDC42 has been found to regulate actin cytoskeleton reorganization and filopodia outgrowth (Nishimura et al. 2005), and to participate in transcriptional regulation via Jun N-terminal kinase/stress activated protein kinase (Minden et al. 1995) and P38 MAPK signaling pathway (Meriane et al. 2000). CDC42 plays important roles in sensing environmental stimuli and transducing signals to regulate cellular processes, including gene expression, cell cycle (Olson et al. 1995),

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vesicle trafficking and cytoskeleton dynamics (Balklava et al. 2007; Cowan and Hyman 2007; Lundquist 2006).

Cellular asymmetry and polarization is crucial for the development of multicellular organisms. In *C. elegans*, one-cell embryo, polarity proteins PAR-1 and PAR-2 distribute in the posterior cortex, whereas PAR-3, PAR-6 and aPKC-3 form a complex localized in the anterior cortex (Hurd and Margolis 2005; Macara 2004). The interaction of CDC42 with PAR-3/PAR-6 and PKC-3 complex is required for asymmetrical distribution of these proteins and embryonic polarity maintenance (Aceto et al. 2006; Gotta et al. 2001; Kay and Hunter 2001). CDC42 is required to remove PAR-2 from the anterior cortex and localize PAR-6 to the anterior cortex (Schonegg and Hyman 2006). The interaction of CDC42 with PAR-3/PAR-6/PKC-3 complex is not only required for endocytic transport (Balklava et al. 2007) but also for diverse cell-type processes including polarized growth of vulval precursors and seam cells, migrations of neuroblasts and axons, and the development of somatic gonad in *C. elegans* (Welchman et al. 2007).

CDC42 regulates the activities of PAR proteins from worm to mammals and flies. In mammals, CDC42 and PAR-6/PAR-3 are involved in the establishment of cell polarity in epithelial cells (Joberty et al. 2000; Johansson et al. 2000) and directing neuroblast cell polarity and asymmetric cell division (Atwood et al. 2007; Lin et al. 2000). In mammalian epithelia, *cdc42* and Par complex are required for apical-basal polarity and junction formation (Yamanaka et al. 2001). During these processes, *cdc42* plays a pivotal role in the establishment of cell polarity by stimulating biogenesis of tight junctions (Black et al. 2007; Mertens et al. 2005). CDC42 recruits Par-6/aPKC to establish cell polarity, and regulates Par-6 activity (Peterson et al. 2004). Human Par-6 binds directly to PKC ζ and forms a stable ternary complex with Rac1 and CDC42 to regulate cell growth (Qiu et al. 2000). It is well known that PAR-3/PAR-6/aPKC complex and CDC42 function together in various cell polarization events including neuron specification, cell migration and front-rear polarization (Nakayama et al. 2008). PAR complex mediates CDC42-induced Rac activation required for neuron polarity establishment (Nishimura et al. 2005; Noda et al. 2001), front-rear polarity of migrating cells and dendritic spine morphogenesis (Schwamborn and Puschel 2004; Sordella and Van Aelst 2008; Solecki et al. 2006). Loss of cell polarity leads to the formation and progression of tumors, and deregulation of the Par complex leads to tumorigenicity (Mertens et al. 2006).

Compared with mammals and *C. elegans*, little information is available on GTPase of parasites. In *Schistosoma*, Rho as a member of the super-family of small GTP-binding proteins has 71–75% identity and approximately 85% similarity with human Rho A, B and C proteins and Rho1 protein specifically expressed in female adult worms (Vermeire et al. 2003). We

previously reported the phylogenetic analysis of *Tscdc42* from *Trichinella spiralis*. *Tscdc42* encodes a small GTPase protein CDC42 with a highly conserved *cdc42* domain, and *Tscdc42* is highly homologous to *C. elegans cdc42* (Yang et al. 2010). Transcriptional analysis revealed that *Tscdc42* was present in the larval and adult worms.

Here, we report that the full-length ORF (open reading frame) of *Tscdc42* was expressed in *E. coli*; the recombinant protein of TsCDC42 was purified and used to raise the polyclonal antibodies. The expression pattern of TsCDC42 in muscle larvae and adult worms of *T. spiralis* was analyzed by western blot. The immuno-localization of TsCDC42 in larvae and adult worms was detected. In addition, cross-species RNAi in *C. elegans* using *Tscdc42* RNAi showed that the expression level of endogenous CDC42 was decreased, which led to the reduction of numbers of progeny in *C. elegans*, suggesting that *Tscdc42* might play the same roles in the early development of *T. spiralis*.

Material and methods

Preparation of the parasites

T. spiralis (ISS 534) has been maintained routinely in the laboratory by serial passages through mice. Kunmin outbred mouse maintained under helminth-free conditions were infected with muscle larvae (L3) of *T. spiralis* by injection of 1,000 larvae through mouth. Six days after infection, adult worms were collected from infected mouse intestine. Newborn larvae were collected from female adult worms incubated in RPMI 1640 medium (GIBCO) for 1 day. Muscle larvae were isolated from the skeletal muscle using standard pepsin digestion method (2 g pepsin and 1 mL 38% HCl in 100 mL ddH₂O) after a 30-day infection. Mouse was housed in the animal room according to the guidelines of the laboratory animal care.

Expression of the recombinant protein and polyclonal antibody production

The cDNA fragment encoding *Tscdc42* was digested from PMD18T-*Tscdc42* with *EcoRI* and *SalI* and cloned into expression vector pET32M (Invitrogen). The resulting plasmid was used to transform competent *E. coli* Dh5 α bacteria. After sequencing confirmation, the plasmid was introduced into *E. coli* BL21 (DE3) competent cells, and the protein expression was induced by the addition of 0.5 mM and 1 mM isopropyl- β -D-thiogalactopyranoside (IPTG) to log-phase bacterial cultures. An aliquot of cells was removed from the culture prior to the induction to serve as a pre-induction control and from an uninduced (without IPTG) culture grown under the same condition. The induced

cultures were grown for 4 h at 37°C with continuous shaking at 220 rpm. Aliquots (1 mL) were withdrawn at 0, 1, 2, 3 and 4 h of induction, and lysates were analyzed on 12% SDS-PAGE. The protein was expressed as a recombinant rTsCDC42, with a N-terminal polyhistidine (His) tag for purification under denaturing condition (8 M urea) using nickel chelation affinity chromatography (Ni-NTA agarose) (QIAGEN, Valencia, CA, USA). Transformed *E. coli* BL21 (DE3) were grown at 37°C to an A_{600} value of 0.6 in LB containing 100- μ g/mL ampicillin and induced with 0.5-mM IPTG for 4 h. The induced cells were disrupted by sonication, and recombinant protein was purified using His Bind purification Kit (Qiagen Novagen) according to the manufacturer's protocol. Antiserum was obtained by injecting mice intraperitoneally with 100 μ g of purified recombinant rTsCDC42, mixed with Freund's incomplete adjuvant (Sigma, St. Louis, MO, USA). After 1 week, the animals were boosted with 100 μ g of protein in Freud complete adjuvant by the same route. After two additional boosters, blood was collected 1 week after the last booster.

SDS-PAGE and western blotting

Crude extracts from muscle larvae and adult worm of *T. spiralis* were prepared by homogenization of the worms in phosphate buffered saline, followed by centrifugation at 10,000 \times g to remove the residual debris. Protein content was determined by Bradford method using a protein assay kit (Biorad). Worm lysates were boiled in Laemmli buffer in the presence of β -mercaptoethanol (β ME) and 10% SDS-PAGE,

and run on mini-Protein II-system (Biorad Inc. Hercules, CA, USA). Proteins were transferred to Immobilon-P polyvinylidene fluoride membrane (Millipore, Bedford, MA, USA) and blocked in TBSTM (25-mM Tris, pH 8.0, 125-mM NaCl, 0.05% Tween 20 (V/V) and 5% non-fat dried milk) for 1 h at room temperature. The membrane was probed with the anti-rTsCDC42 polyclonal mouse anti-sera at 1:500 or monoclonal anti-tubulin (1:500, KPL) and goat anti-mouse antibody conjugated to horseradish peroxidase at (1:3,000, KPL) for 30 min. Chemiluminescent detection was performed with the ECL Reagent (Biorad).

Immunostaining of adult worm and muscle larvae of *T. spiralis*

Muscle larvae and adult worms were collected from infected mouse muscle and intestine, respectively. After PBS wash, the worms were immunostained with the anti-rTsCDC42 antibody using protocol adapted from *C. elegans* (Bowerman et al. 1993; Crittenden et al. 1994; Hunter and Kenyon 1996). Briefly, the worms were spread on polylysine coated slides. The female adult worms were cut at the middle to release embryos and larvae onto the slides. After removing the liquid from the slides, a cover slip was placed on worms. The slides were frozen in liquid nitrogen and immersed in fixative solution (4% formaldehyde 10 min, cold 100% MeOH 5 min) after removing the cover slip. The slides were washed in PBS for 10 min, then incubated in block solution (PBS containing 1%BSA) for 1 h, and stained with primary antibody (1:500 diluted in

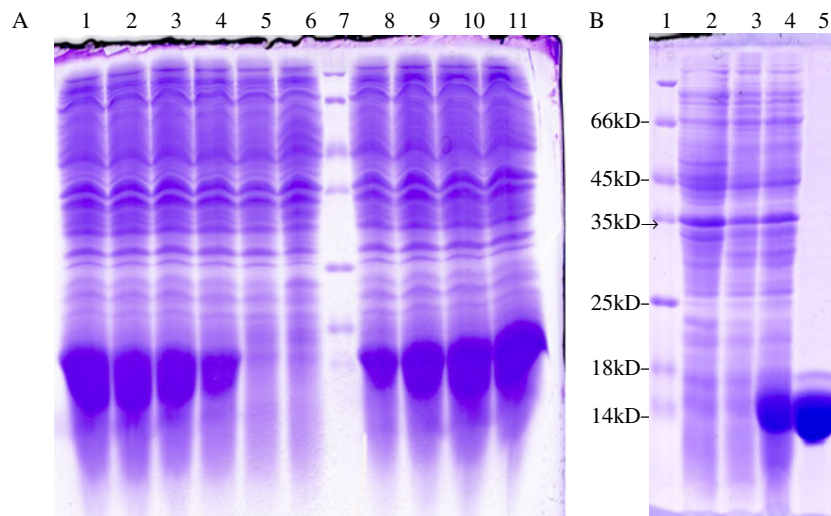


Fig. 1 Recombinant TsCdc42 was successfully produced in *E. coli*. **a** SDS-PAGE analysis of *E. coli* BL21 (transformed by pETTsdc42) total cell lysates induced with different concentrations of IPTG. Lanes 1–5 represented 0.5-mM IPTG induced for 4 h (1), 3 h (2), 2 h (3), 1 h (4), and pre-induction with IPTG (5), respectively. Lane 6 represented *E. coli* BL21 cell lysate, and lane 7 was standard Protein Marker (Fermentas). Lanes 8–11 represented total cell lysates from *E. coli*

BL21 transformed with pETTsdc42 and induced with 1.0-mM IPTG 1 h (8), 2 h (9), 3 h (10) and 4 h (11), respectively. **b** Purification of recombinant TsCDC42 analyzed on SDS-PAGE. Lane 1, Protein Marker; Lane 2, cell lysates of *E. coli* BL21; Lane 3, cell lysates of pETTsdc42 transformed *E. coli* BL21 before addition of IPTG; Lane 4, cell lysates of pETTsdc42 transformed *E. coli* BL21 induced with 0.5-mM IPTG 4 h; Lane 5, purified TsCDC42

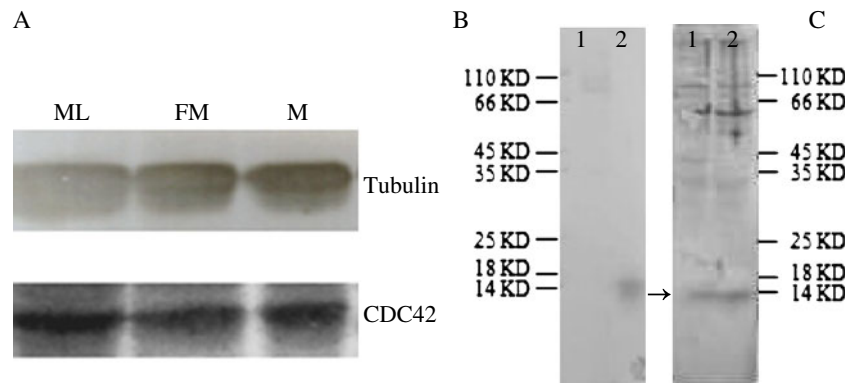


Fig. 2 The expression of TsCDC-42 in different stages of *T. spiralis* detected using the antibody against recombinant TsCDC42 protein. **a** Lanes ML, FM and M were crude extracts from muscle larvae, female and male adult worms, respectively. Upper lanes were probed with anti-tubulin as protein-loading control, and lower lanes represented

the CDC42 protein level. **b** Crude extracts from muscle larvae (lane 1) and recombinant protein (lane 2) detected with pre-immune serum as the negative control. **c** Crude extracts from newborn larvae (lane 1) and muscle larvae (lane 2) detected with the anti-rTsCDC42 antibody by western blot. Arrow showed the CDC42 band

block solution) for 2 h. The slides were washed three times in PBST (5/50/15 min each) and stained with secondary antibody for 2 h (cy3-conjugated goat anti-mouse-IgG 1:500 in block solution). After 4× washes in PBST, the slides were mounted with 70% glycerol for microscopy.

Functional analysis of *Tscdc42* using RNA interference in *C. elegans*

Functional analysis of *Tscdc42* was carried out through knockdown *cdc42* homologous gene expression in *C. elegans*. *Tscdc42* was released from PMD18-TsCDC42 plasmid after digestion with *Xba*I and *Pst*I, and ligated with L4440 vector digested with the same enzymes. The resulting plasmid L4440-TsCDC42 was sequenced to confirm its identity and then transformed into HT115 competent bacteria. A single colony from each RNAi clone was grown in LB broth containing 50-mg/mL ampicillin. The RNAi bacteria were then seeded onto NGM plates containing 1-mM isopropylthiogalactoside and 50-mg/mL ampicillin. Seeded plates were dried overnight at room temperature and then kept at 4°C for subsequent use. Cross-species RNAi was performed using RNAi feeding protocol (Kamath et al. 2001). Synchronized L1 larvae of *C. elegans* wild-type N2 worm were grown on RNAi and control plates. Western blot was used to detect the endogenous expression level of CDC42 in *C. elegans* feeding with normal OP50 bacteria, control RNAi food (empty L4440 vector transformed HT115 bacteria) and *Tscdc42* RNAi bacterial food (L4440-TsCDC42 transformed HT115). The ability of egg production and hatching rate of *C. elegans* wild-type worm feeding with *Tscdc42* RNAi bacterial food and L4440 empty vector transformed HT115 bacterial as a control were measured. Two parallel groups of *Tscdc42* RNAi worms along with one group of RNAi control worms were performed. Each group consisted of 20 new adult worms. The eggs and progeny of 20

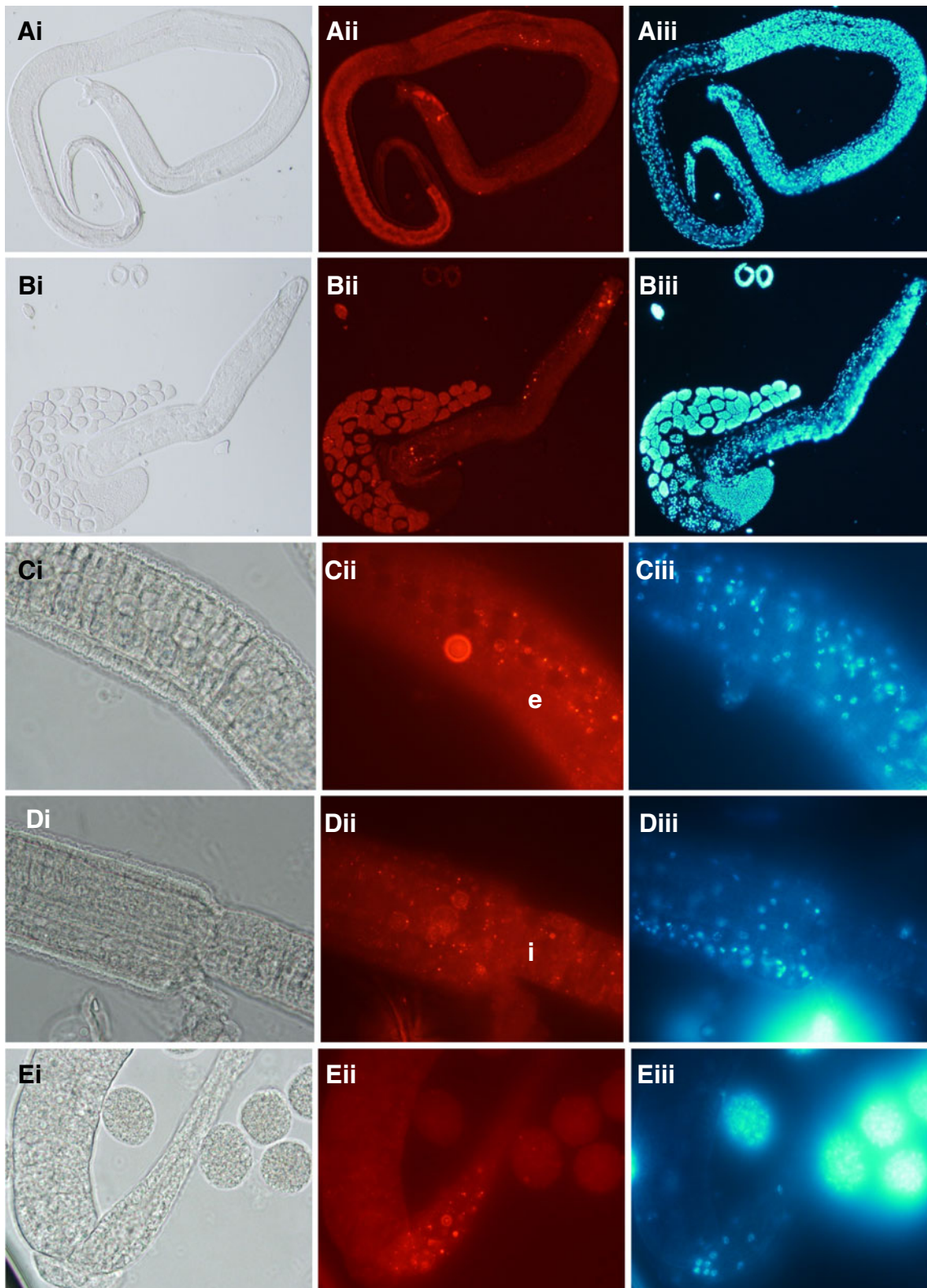
adult worms produced in the following 4 days were counted after feeding with RNAi foods. The adult worms were moved to new RNAi plates every 2–4 h; the produced eggs were counted immediately and hatched larvae were counted after 6–8 h. The numbers of F1 adult worms were counted on the following day. The experiments were repeated three times. The data were analyzed using SPSS 10 statistics software.

Results

Expression of the recombinant TsCDC42 protein and purification

To obtain large quantities of TsCDC42 protein for immunological and functional studies, its ORF was cloned into pET-32m vector which digested with *Eco*R I and *Sal* I resulting in the expression vector pET-TsCDC42. The construction was confirmed using restriction enzyme

Fig. 3 Localization of TsCDC42 in various stages of *T. spiralis*. **A**, male; **B–E**, female adult worm; **F–G**, embryos; **H**, newborn larvae; **I**, muscle larvae; and **L**, female adult worm staining with pre-immune mouse serum as negative control. Samples were fixed and incubated with polyclonal anti-TsCDC42 mouse serum and DAPI; antibody binding was detected by using cy3 fluor-conjugated anti-mouse immunoglobulin. Red fluorescence was observed in the stichocytes of esophagus, intestine and rectum of male adult worm (**Aii**), embryos (**Bii**), the stichocytes of esophagus (**Cii**), intestine (**Dii**) and the forepart of esophagus of female adult worm (**Eii**) as well as the midterm embryos (**Eii**, **Fii**), early (arrows) and late stage (open arrow head) embryos (**Gii**) and newborn larvae (**Hii**). Red fluorescence also present in the stichocytes of esophagus, intestine, gonad primordial and tail of muscle larvae (**Iii**). Panels **Ai**, **Bi**, **Ci**, **Di**, **Ei**, **Fi**, **Gi**, **Hi**, **Ii** and **Li** are phase micrographs and panels **Aii**, **Bii**, **Cii**, **Dii**, **Eii**, **Fii**, **Gii**, **Hii** and **Iii** are fluorescence microscopy micrographs from staining using anti-TsCDC42 antibodies or pre-immune mouse serum (**Lii**). Panels **Aiii**, **Biii**, **Ciii**, **Diii**, **Eiii**, **Fiii**, **Giii**, **Hiii**, **Iiii** and **Liii** are DAPI-stained fluorescence microscopy micrographs. **in** intestine; **e** esophagus



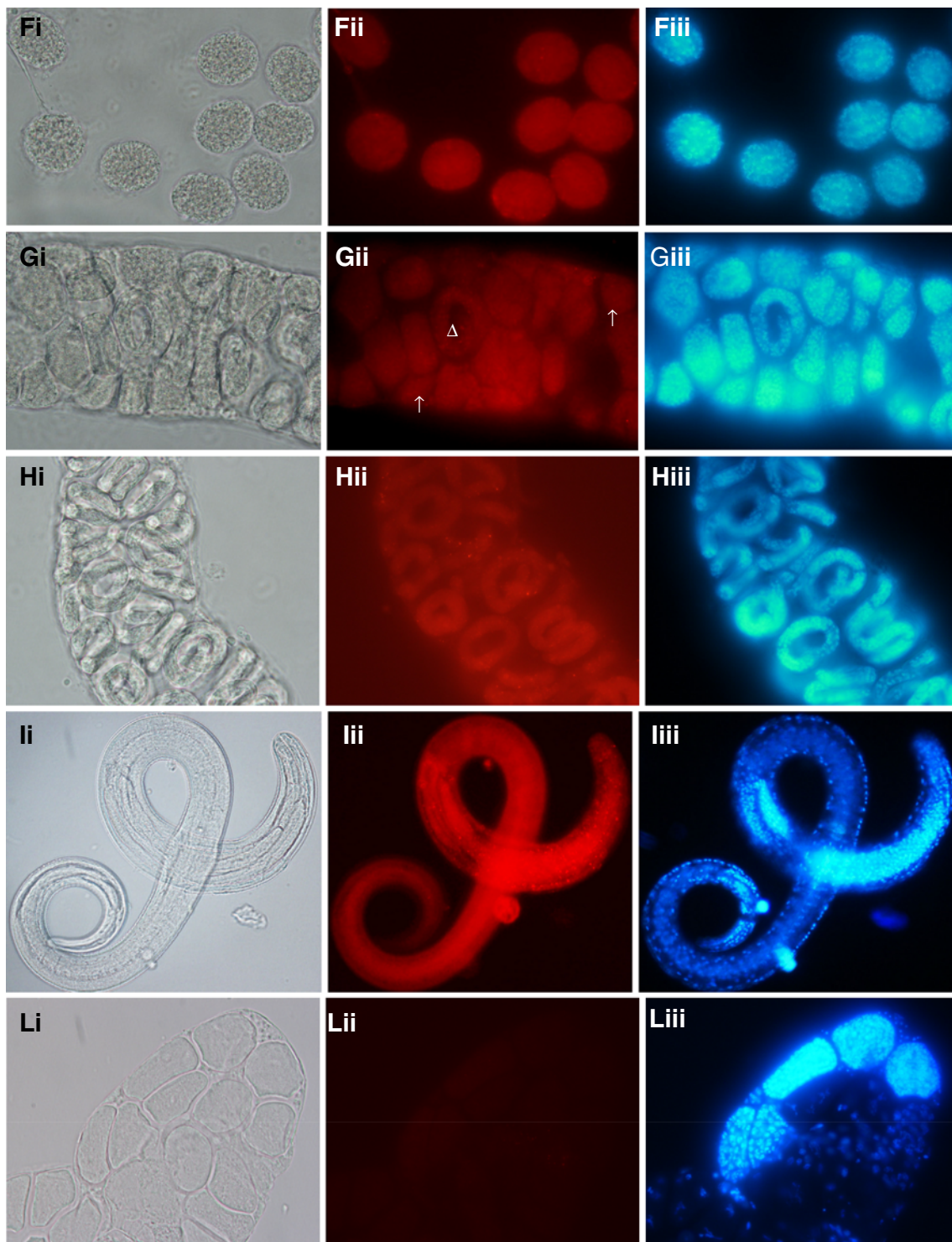


Fig. 3 (continued)

analysis and nucleotide sequencing. The cell extracts from transformed *E. coli* BL21 cultures induced with IPTG showed a major protein band approximately 16 kDa in SDS-PAGE analysis (Fig. 1a). The purified recombinant Tscdc42 (Fig. 1b) was then used to raise antibodies in mice for western blot and immunostaining.

Expression pattern of TsCDC42 in different developmental stages of *T. spiralis*

To determine the expression pattern during development, the protein level of *Tscdc42* in different stages of *T. spiralis* was detected using antibodies against recombinant TsCDC42.

Crude extracts of muscle larvae, male and female adult worms were analyzed on western blot. The results showed that anti-rTsCDC42 serum positively identified a ~16-kDa band in the lysates from newborn larvae, muscle larvae and both male and female adult worms of *T. spiralis* (Fig. 2a). The pre-immune serum could not detect any specific band in this area in the extracts of muscle larvae (Fig. 2b) compared with newborn larvae and muscle larvae detected with anti-rTsCDC42 positive serum (Fig. 2c).

Immuno-localization of TsCDC42 in muscle larvae and adult stage of *T. spiralis*

Muscle larvae, male and female adult worms of *T. spiralis* were incubated with anti-rTsCDC42 antibodies, and TsCDC42 was found to be present in the adult worm and muscle larvae as well as the embryos released from female adult worms.

In male adult worm, red fluorescence was observed in the stichocytes of esophagus, intestine and rectum (Fig. 3Aii). Strong fluorescence signals were present in the embryos of *T. spiralis* released from mature female adult worm (Fig. 3Bii).

Red fluorescence was also observed in the stichocyte phase of esophagus of adult female worms (Fig. 3Cii), intestine (Fig. 3Dii) and the forepart of esophagus before stichocyte phase of female adult worms (Fig. 3Eii).

The staining of TsCDC42 was also observed in the embryos of midterm (Fig. 3Eii, Fii), late (Fig. 3Gii) and early stages (Fig. 3Gii). The TsCDC42 was also strongly expressed in newly formed newborn larvae within the mature female uterus (Fig. 3Hii).

In muscle larvae, red fluorescence was also observed in the stichocytes of esophagus, intestine, intense staining of gonad primordium and tail of muscle larvae (Fig. 3Iii). No fluorescence was detected in worms stained with pre-immune mouse serum as control (Fig. 3Lii).

The results of immuno-localization showed that TsCDC42 was expressed at higher level in muscle larvae stage than in the adult worms, except the quite high-level expression in the embryos inside female adult worm body.

Cross-species RNAi by *Tscdc42* RNAi in *C. elegans* decrease the number of progeny in *C. elegans*

Since the sequence of TsCDC42 showed high identity to *C. elegans* CDC42 (in 62%), RNAi in *Trichinella* was not developed up to now. In order to know the function of *Tscdc42*, cross-species RNAi was performed using *Tscdc42* RNAi in *C. elegans* to see whether it could knock down *C. elegans* *cdc42* function. Since *cdc42* mutant in *C. elegans* showed embryonic lethal and fertility reduction, the progeny from two groups of worms feeding with *Tscdc42* RNAi bacteria (20 worms each group) was counted either with control bacteria (L4440 empty vector).

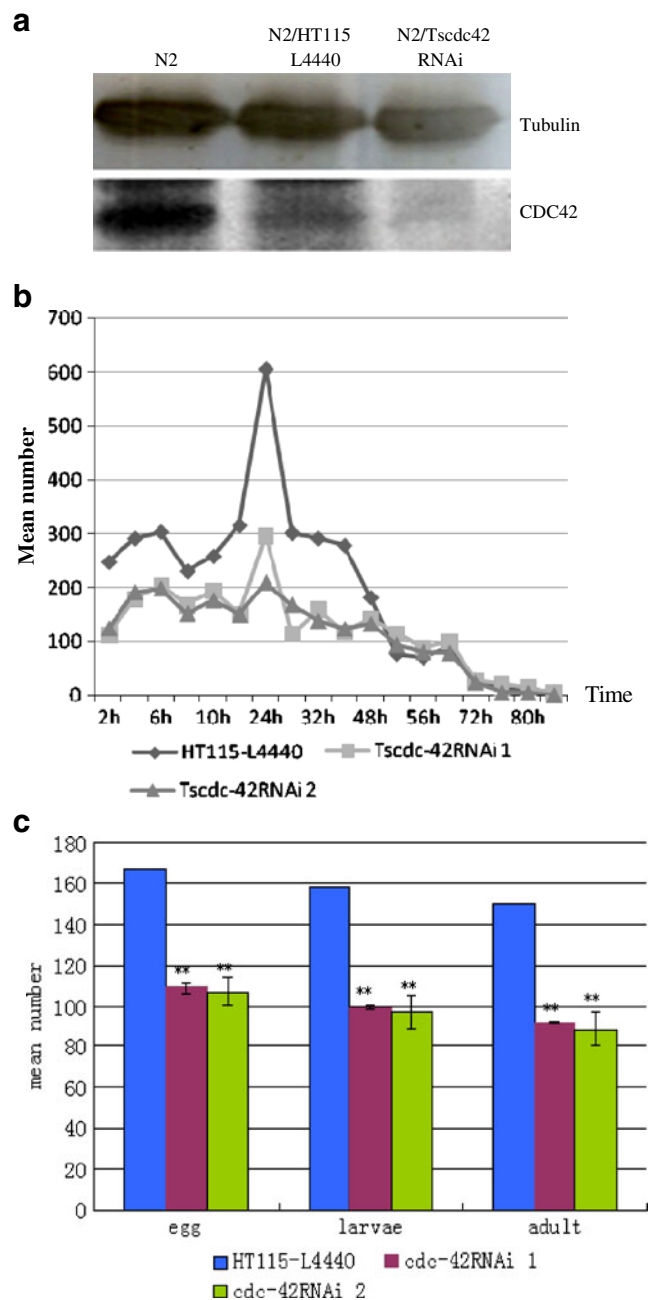


Fig. 4 Cross-species RNAi in *C. elegans* using *Tscdc42* RNAi reduced the expression level of the endogenous CDC-42 in *C. elegans* (a) and decreased the progeny counts in *C. elegans* (b, c). **a** The endogenous protein level of CDC42 in *C. elegans* wild-type and RNAi worms detected by western blot using anti-TsCDC42 antibody. Lane N2, crude extracts from mixed stages of wild type; Lane N2/HT115 (L4440), extracts from mixed stages of wild-type N2 feeding on HT115 transformed with L4440 empty vector. Lane N2/*Tscdc42* RNAi, crude extracts from N2 feeding with *Tscdc42* RNAi bacteria food. **b** Progeny from 20 adult worms of *C. elegans* wild-type N2 feeding with control bacteria (HT115 transformed with L4440 empty vector) and *Tscdc42* RNAi bacteria (two groups). **c** The bars represent the mean numbers of eggs, hatching larvae and F1 adult worms produced by 20 adult worms either fed on control or *Tscdc42* RNAi bacteria (two groups). Error bars represent standard error of the mean

The endogenous expression of CDC42 in *C. elegans* was detected using the anti-rTsCDC42 antibody. Fig. 4a showed the endogenous expression level of CDC42 in *C. elegans* wild-type N2 worm feeding with *E. coli* OP50 as normal food; CDC42 expression level was reduced in *Tscdc42* RNAi worms whereas in RNAi control worm, the CDC42 expression level was not affected, though the intensity of the CDC42 band was not as strong as N2 worms feeding with OP50 that might represent the RNAi toxicity in the worms.

Consistent with the results showing lower level expression of CDC42 in *C. elegans Tscdc42* RNAi worm, reduction of progeny in *Tscdc42* RNAi worms was observed. The ratio of F1 larval hatching (Fig. 4b) and the number of F1 eggs developed into adult worms were also decreased significantly (Fig. 4c). The results of cross-species of *Tscdc42* RNAi in *C. elegans* suggested that *Tscdc42* might play the same function in *Trichinella* as *cdc42* in *C. elegans*.

Discussion

In this paper, we reported the expression of recombinant *T. spiralis* TsCDC42 in *E. coli*, the expression level and localization of TsCDC42 in different stages of *T. spiralis* and functional characterization of the *cdc42* gene in *C. elegans*. Our previous work showed that the predicted TsCDC42 open reading frame encodes a 147 amino acid protein (Yang et al. 2010). TsCDC42 displays a high level of identity with the Rho-family GTPase protein CDC42 and has the highest identity with the CDC42 from *Drosophila* (67%). The sequence identity of TsCDC42 with the *C. elegans* CDC42 homologue is 62%. The western blots showed that TsCDC42 was found in muscle larvae and in male and female adult worms. The immuno-localization of TsCDC42 revealed that TsCDC42 was abundantly distributed in the muscle worm, female and male adult worms, the newborn larvae as well as the embryo.

As a signaling transduction molecule, *cdc42* plays an important role in cell polarity, cytoskeleton arrangement and cell motility. Although there are a lot of reports on the functions of CDC42 in mammals, *Drosophila* and *C. elegans*, little information is available in parasitic nematodes. The similarity and identity of TsCDC42 with other CDC42 protein is high (up to 70%). In *C. elegans*, PAR-3, PAR-6 and PKC-3 form a complex to regulate the polarity of one-cell embryo (Macara 2004). The interaction of CDC42 with PAR-6 is required for PAR asymmetry maintenance and one-cell embryo polarity (Aceto et al. 2006). Since most of PAR proteins are maternal, strong maternal reduction of *cdc42* by RNAi causes defects in the first cell division and embryonic lethality (Gotta et al. 2001). The phenotype of *cdc42* RNAi is similar to *par-3*, *par-6* and *pkc-3* mutants, and results in multiple ventral protrusion in adult progeny, a characteristic

of a multiple vulva (Muv) phenotype (Welchman et al. 2007). The expression of CDC42 in *C. elegans* is developmentally regulated with the highest level at the embryonic stage and decreasing progressively during development except an increase at the L3 stage (Chen et al. 1993).

The expression of TsCDC42 in different stages of *T. spiralis* showed that TsCDC42 was ubiquitously expressed in muscle larvae stage and adult worms. The immuno-localization results were consistent with this. The immuno-localization showed that TsCDC42 was persistently expressed in muscle larvae stage and adult worms, except the quite high-level expression in the embryos within female adult worm uterus. TsCDC42 followed a developmental pattern similar to CDC42 in *C. elegans*, with a high expression level in the embryos, suggesting related roles in the early development of the parasite. Since the sequence of TsCDC42 showed high identity to *C. elegans* CDC42 (in 62%), and RNAi technology has not developed for *Trichinella* up to now, cross-species RNAi in *C. elegans* was performed to investigate the function of *Tscdc42* to see whether it could knock down the *C. elegans* orthologs. The results revealed that *Tscdc42* RNAi in *C. elegans* could knock down endogenous CDC42 expression level in *C. elegans* and reduce the progeny and eggs counts in RNAi worms compared to control. The larval hatching rate and the numbers of F1 eggs that developed into adult worms were decreased significantly. The heterospecies of RNAi suggests that *Tscdc42* is functional and might play a role in the early development of parasite.

In several previous studies, cross-species of RNAi has been used to investigate the gene function (Gao et al. 2006; Lendner et al. 2008), but this approach has not been applied in *Trichinella*. Although the entire genomic sequencing of *Trichinella* has been finished, a lot of gene function remains unclear. In *C. elegans*, the RNAi techniques have been well-established, using the cross-species RNAi in *C. elegans* that could accelerate the identification of gene function in parasite nematodes. Our results demonstrated the value of cross-species RNAi as a tool for the functional identification of *Trichinella* genes and can be applied in the functional study of genes in the other parasitic nematodes. Further study will be focused on the role of TsCDC42 in signaling pathway and other molecules in this pathway.

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