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Research Brief

Cloning, sequencing and phylogenetic analysis of the small GTPase gene *cdc-42* from *Ancylostoma caninum*

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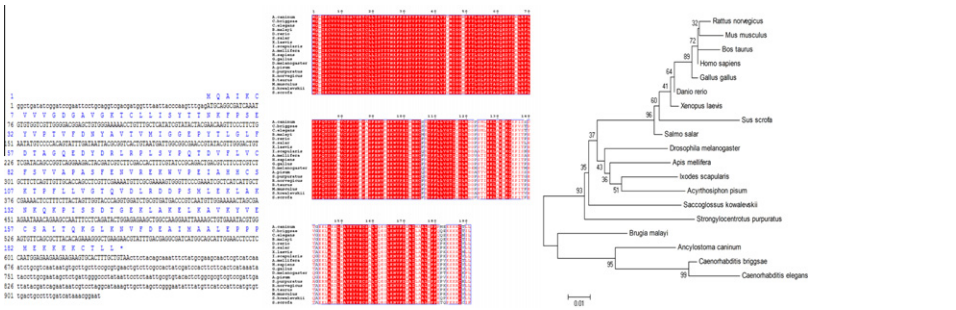
HIGHLIGHTS

- A small GTPase *cdc-42* was cloned from parasitic nematode *Ancylostoma caninum*.
- The ORF of Accdc-42 contains 191 amino acids residues with a cdc-42 domain.
- Phylogenetic analyses revealed that *Accdc-42* was highly conserved.
- *Accdc-42* was expressed in L1/L2, L3 larvae and adult worm of *A. caninum*.

GRAPHICAL ABSTRACT



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ABSTRACT

CDC-42 is a member of the Rho GTPase subfamily that is involved in many signaling pathways, including mitosis, cell polarity, cell migration and cytoskeleton remodeling. Here, we present the first characterization of a full-length cDNA encoding the small GTPase *cdc-42*, designated as *Accdc-42*, isolated from the parasitic nematode *Ancylostoma caninum*. The encoded protein contains 191 amino acid residues with a predicted molecular weight of 21 kDa and displays a high level of identity with the Rho-family GTPase protein CDC-42. Phylogenetic analysis revealed that *Accdc-42* was most closely related to *Caenorhabditis briggsae cdc-42*. Comparison with selected sequences from the free-living nematode *Caenorhabditis elegans, Drosophila melanogaster, Xenopus laevis, Danio rerio, Mus musculus* and human genomes showed that *Accdc-42* is highly conserved. AcCDC-42 demonstrates the highest identity to CDC-42 from *C. briggsae* (94.2%), and it also exhibits 91.6% identity to CDC-42 from *C. elegans* and 91.1% from *Brugia malayi*. Additionally, the transcript of *Accdc-42* was analyzed during the different developmental stages of the worm. *Accdc-42* was expressed in the L1/L2 larvae, L3 larvae and female and male adults of *A. caninum*.

1. Introduction

Small GTPases of the Rho family are key players in cytoskeleton remodeling and cell polarity in different systems (Lundquist, 2006), and they function as GDP/GTP-regulated molecular switches. Signal transduction through GTPases requires regulated cycling of the GTPases between the GTP-bound active state and the GDP-bound inactive state. A GTP-bound Rho GTPase can bind to various effectors to elicit different biological activities. Rho GTPases are regulated by two classes of enzymes: the GTP-bound state is activated by guanine nucleotide exchange factors (GEFs) and inactivated by GTPase activating proteins (GAPs), leading to the GDP-bound inactive state. GEFs stimulate the exchange of GDP for GTP on GTPases, whereas GAPs inhibit GTPases by potentiating their intrinsic GTPase activity (Lundquist, 2006).

CDC-42 is a member of the Rho GTPase subfamily that is involved in many processes, including cell polarity, cell migration

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Fig. 1. Sequence of the *Accdc-42* cDNA from *A. caninum*. The initiator codon and the stop codon are shown, including the position of the 5′ untranslated region (UTR) and the 3′ UTR. The amino acid translation of the coding DNA is shown on the upper side in signal letter code.

and cytoskeleton remodeling (Kim, 2000). In *Caenorhabditis elegans*, CDC-42 interacts with the PAR-3/PAR-6/PKC-3 complex, which is involved in polarity maintenance and cell migration (Aceto et al., 2006). CDC-42 organizes embryonic polarity by controlling the localization and activity of PAR proteins (Kay and Hunter, 2001). The interaction of CDC-42 with the PAR-3/PAR-6/PKC-3 complex is conserved throughout animal evolution (Lin et al., 2000; Noda et al., 2001; Solecki et al., 2006) and allows these proteins to function together in diverse cell types (Welchman et al., 2007). In mammalian cells, CDC-42 localizes to cell-cell junctions and is crucial for apico-basal polarity in epithelial cells (Yamanaka et al., 2001). In developing neurons, the PAR-3/PAR-6/PKC-3/CDC-42 complex is required to specify the fate of neurons and maintain the polarity of axons (Cappello et al., 2006; Schwamborn and Puschel, 2004; Solecki et al., 2006). The function of CDC-42 and its interaction with PAR complex has been investigated thoroughly in mammalian cells, *Xenopus*, *Drosophila* and *C. elegans*. However, unlike the free-living nematode *C. elegans*, the function of *cdc-42* in hookworms remains unknown.

Hookworms are important parasitic nematodes that can parasitize in human and animal intestines. The canine hookworm *Ancylostoma caninum* parasitizes in the intestines of dogs and sucks blood from the intestine wall. In addition to its veterinary importance, *A. caninum* can also cause zoonotic disease in humans. The larvae of *A. caninum* hatch from eggs and develop into infective larvae via two moltings. The infective larvae then infect host animals such as dogs and cats, migrate into the intestine and develop into adult worms following two more moltings. If the infective larvae invade humans, they can cause cutaneous larvae migrans (CLM) or "creeping eruptions," which are hypersensitivity reactions in response to the migration of *A. caninum* larvae, though they cannot develop into adult worms just by migrating under the skin.

Here, we report the isolation of complementary DNA (cDNA) encoding the small GTPase CDC-42 from *A. caninum* by RT-PCR. The conceptual translation of *cdc-42* indicates that this cDNA encodes a protein of 191 amino acids. We also conducted the phylogenetic analysis of *cdc-42* from different species. Finally, RT-PCR was performed to detect *Accdc-42* in different stages of *A. caninum* for transcriptional analysis, and we found that *Accdc-42* was expressed in L1/L2 larvae, L3 larvae and adult worms of *A. caninum*.

2. Material and methods

2.1. Parasite infection and recovery

A. caninum has been maintained in the lab by serial passages in dogs since 2005. Fecal cultures from infected dogs were incubated at room temperature to recover first-stage larvae after one day of culture in ddH_2O, while second–stage larvae (L2) were recovered after 3 days of culture, and infective third stage larvae (L3) were recovered after 6–7 days of culture (Yang et al., 2009, 2011). L3 larvae were used to infect dogs. Adult worms were collected at necropsy from the intestines of infected dogs one month after inoculation with 3000–5000 L3 via the skin. Nematodes at each stage were recovered and resuspended in phosphate-buffered saline (PBS) and were then washed extensively to remove any debris and subsequently frozen at −70 °C before RNA extraction. Dogs were housed in animal rooms according to laboratory animal care guidelines, and the study was approved by XMU (approval No. 2010081808) and conducted with adherence to the guidelines of XMU for animal husbandry.

2.2. RNA extraction

Adult worm mRNA was extracted using the Trizol (MRC) method following the protocol of the manufacturer (MRC). The precipitated RNA pellet was resuspended in 30 μL of RNase-free DEPC-treated water. In addition, 5 μL of RNA was run on a 1% agarose gel. Adult worm cDNA was prepared using the RevertAid First Strand cDNA Synthesis Kit (Fermentas) with the oligo (dT) primer following the instructions described by the manufacturer.

2.3. Isolation of a full-length cDNA encoding a small GTPase protein cdc-42 from A. caninum and cloning

A small GTPase *cdc-42* cDNA fragment of *A. caninum* was obtained by RT-PCR using the adult worm cDNA as a template. Acdc42F (5′-CCGTTTATGATCAAAGGCAGT) and Acdc42R (5′-AGAATAAACAGAAGCCAATTTCC) were designed according to the EST sequence (accession No. FC550695) and, in addition to SL1 (5′-GGTTTAATTACCCAAGTTTGAG-3′) (Hough et al., 1999), were synthesized (Shanghai Sangon Co.). The PCR mixture consisted of